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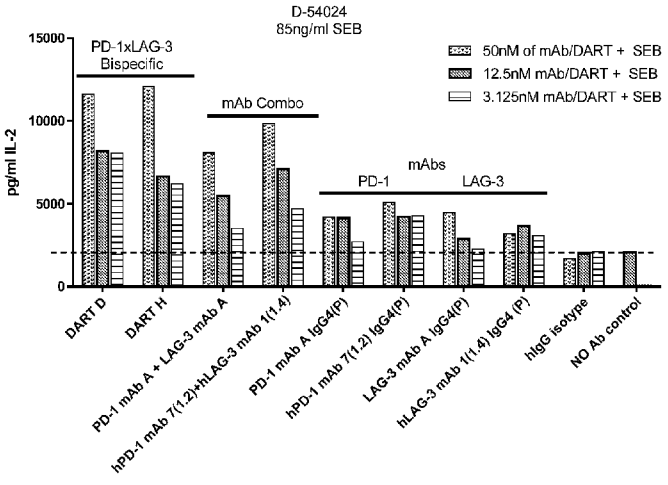
(54) Title: PD-1-BINDING MOLECULES AND METHODS USE THEREOF

Figure 19



(57) Abstract: The present invention is directed to selected anti-PD-1 antibodies capable of binding to both cynomolgus monkey PD-1 and to human PD-1 : PD-1 mAb 1, PD-1 mAb 2, PD-1 mAb 3, PD-1 mAb 4, PD-1 mAb 5, PD-1 mAb 6, PD-1 mAb 7, PD-1 mAb 8, PD-1 mAb 9, PD-1 mAb 10, PD-1 mAb 11, PD-1 mAb 12, PD-1 mAb 13, PD-1 mAb 14, or PD-1 mAb 15, and to humanized and chimeric versions of such antibodies. The invention additionally pertains to PD-1 -binding molecules that comprise PD-1 binding fragments of such anti-PD-1 antibodies, immunoconjugates, and to bispecific molecules, including diabodies, BiTEs, bispecific antibodies, etc., that comprise (i) such PD-1 -binding fragments, and (ii) a domain capable of binding an epitope of a molecule involved in regulating an immune check point present on the surface of an immune cells. The present invention also pertains to methods of using molecules that bind PD-1 for stimulating immune responses, as well as methods of detecting PD-1.

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Title Of The Invention:

PD-1-Binding Molecules and Methods of Use Thereof

Cross-Reference to Related Applications

[0001] This application claims priority to U.S. Patent Applications Serial No. 62/198,867 (filed on July 30, 2015; pending), 62/239,559 (filed on October 9, 2015; pending), 62/255,140 (filed on November 13, 2015; pending), and 62/322,974 (filed on April 15, 2016; pending), each of which applications is herein incorporated by reference in its entirety.

Reference To Sequence Listing

[0002] This application includes one or more Sequence Listings pursuant to 37 C.F.R. 1.821 et seq., which are disclosed in computer-readable media (file name: 1301_0122PCT_Sequence_Listing_ST25.txt, created on July 1, 2016, and having a size of 282,789 bytes), which file is herein incorporated by reference in its entirety.

Field Of The Invention

[0003] The present invention is directed to PD-1 binding molecules that comprise the PD-1-binding domain of selected anti-PD-1 antibodies capable of binding to both cynomolgus monkey PD-1 and to human PD-1: PD-1 mAb 1, PD-1 mAb 2, PD-1 mAb 3, PD-1 mAb 4, PD-1 mAb 5, PD-1 mAb 6, PD-1 mAb 7, PD-1 mAb 8, PD-1 mAb 9, PD-1 mAb 10, PD-1 mAb 11, PD-1 mAb 12, PD-1 mAb 13, PD-1 mAb 14, or PD-1 mAb 15. The invention particularly concerns PD-1 binding molecules that are humanized or chimeric versions of such antibodies, or that comprise PD-1 binding-fragments of such anti-PD-1 antibodies (especially immunocongugates, diabodies, BiTEs, bispecific antibodies, *etc.*). The invention particularly concerns such PD-1-binding molecules that are additionally capable of binding an epitope of a molecule involved in regulating an immune check point that is present on the surface of an immune cell. The present invention also pertains to methods of using such PD-1-binding molecules to detect PD-1 or to stimulate an immune response. The present invention also pertains to methods of combination therapy in which a PD-1-binding molecule that comprises one or more PD-1-binding domain(s) of such selected anti-PD-1 antibodies is administered in combination with one or more additional molecules that are effective in stimulating an immune response and/or in combination with one or more additional molecules that specifically bind a cancer antigen.

Background Of The Invention

I. Cell Mediated Immune Responses

[0004] The immune system of humans and other mammals is responsible for providing protection against infection and disease. Such protection is provided both by a humoral immune response and by a cell-mediated immune response. The humoral response results in the production of antibodies and other biomolecules that are capable of recognizing and neutralizing foreign targets (antigens). In contrast, the cell-mediated immune response involves the activation of macrophages, Natural Killer cells (NK), and antigen specific cytotoxic T-lymphocytes by T-cells, and the release of various cytokines in response to the recognition of an antigen (Dong, C. *et al.* (2003) "*Immune Regulation by Novel Costimulatory Molecules*," Immunolog. Res. 28(1):39-48).

[0005] The ability of T-cells to optimally mediate an immune response against an antigen requires two distinct signaling interactions (Viglietta, V. *et al.* (2007) "*Modulating Co-Stimulation*," Neurotherapeutics 4:666-675; Korman, A.J. *et al.* (2007) "*Checkpoint Blockade in Cancer Immunotherapy*," Adv. Immunol. 90:297-339). First, antigen that has been arrayed on the surface of Antigen-Presenting Cells (**APC**) must be presented to an antigen-specific naive $CD4^+$ T-cell. Such presentation delivers a signal via the T-Cell Receptor (**TCR**) that directs the T-cell to initiate an immune response that will be specific to the presented antigen. Second, a series of costimulatory and inhibitory signals, mediated through interactions between the APC and distinct T-cell surface molecules, triggers first the activation and proliferation of the T-cells and ultimately their inhibition. Thus, the first signal confers specificity to the immune response whereas the second signal serves to determine the nature, magnitude and duration of the response.

[0006] The immune system is tightly controlled by costimulatory and co-inhibitory ligands and receptors. These molecules provide the second signal for T-cell activation and provide a balanced network of positive and negative signals to maximize immune responses against infection while limiting immunity to self (Wang, L. *et al.* (March 7, 2011) "*VISTA, A Novel Mouse Ig Superfamily Ligand That Negatively Regulates T-Cell Responses*," J. Exp. Med. 10.1084/jem.20100619:1-16; Lepenies, B. *et al.* (2008) "*The Role Of Negative Costimulators During Parasitic Infections*," Endocrine, Metabolic & Immune Disorders - Drug Targets 8:279-288). Of particular importance is binding between the B7.1 (CD80) and B7.2 (CD86) ligands of the Antigen-Presenting Cell and the CD28 and CTLA-4 receptors of the CD4+ T

lymphocyte (Sharpe, A.H. *et al.* (2002) "*The B7-CD28 Superfamily*," Nature Rev. Immunol. 2:116-126; Dong, C. *et al.* (2003) "*Immune Regulation by Novel Costimulatory Molecules*," Immunolog. Res. 28(1):39-48; Lindley, P.S. *et al.* (2009) "*The Clinical Utility Of Inhibiting CD28-Mediated Costimulation*," Immunol. Rev. 229:307-321). Binding of B7.1 or of B7.2 to CD28 stimulates T-cell activation; binding of B7.1 or B7.2 to CTLA-4 inhibits such activation (Dong, C. *et al.* (2003) "*Immune Regulation by Novel Costimulatory Molecules*," Immunolog. Res. 28(1):39-48; Lindley, P.S. *et al.* (2009) "*The Clinical Utility Of Inhibiting CD28-Mediated Costimulation*," Immunol. Rev. 229:307-321; Greenwald, R.J. *et al.* (2005) "*The B7 Family Revisited*," Ann. Rev. Immunol. 23:515-548). CD28 is constitutively expressed on the surface of T-cells (Gross, J., *et al.* (1992) "*Identification And Distribution Of The Costimulatory Receptor CD28 In The Mouse*," J. Immunol. 149:380–388), whereas CTLA-4 expression is rapidly upregulated following T-cell activation (Linsley, P. *et al.* (1996) "*Intracellular Trafficking Of CTLA4 And Focal Localization Towards Sites Of TCR Engagement*," Immunity 4:535–543). Since CTLA-4 is the higher affinity receptor (Sharpe, A.H. *et al.* (2002) "*The B7-CD28 Superfamily,*" Nature Rev. Immunol. 2:116-126), binding first initiates T-cell proliferation (via CD28) and then inhibits it (via nascent expression of CTLA-4), thereby dampening the effect when proliferation is no longer needed.

[0007] Further investigations into the ligands of the CD28 receptor have led to the identification and characterization of a set of related B7 molecules (the "B7 Superfamily") (Coyle, A.J. *et al.* (2001) "*The Expanding B7 Superfamily: Increasing Complexity In Costimulatory Signals Regulating T-Cell Function*," Nature Immunol. 2(3):203-209; Sharpe, A.H. *et al.* (2002) "*The B7-CD28 Superfamily*," Nature Rev. Immunol. 2:116-126; Greenwald, R.J. *et al.* (2005) "*The B7 Family Revisited*," Ann. Rev. Immunol. 23:515-548; Collins, M. *et al.* (2005) "*The B7 Family Of Immune-Regulatory Ligands*," Genome Biol. 6:223.1-223.7; Loke, P. *et al.* (2004) "*Emerging Mechanisms Of Immune Regulation: The Extended B7 Family And Regulatory T-Cells*." Arthritis Res. Ther. 6:208-214; Korman, A.J. *et al.* (2007) "*Checkpoint Blockade in Cancer Immunotherapy*," Adv. Immunol. 90:297-339; Flies, D.B. *et al.* (2007) "*The New B7s: Playing a Pivotal Role in Tumor Immunity*," J. Immunother. 30(3):251-260; Agarwal, A. *et al.* (2008) "*The Role Of Positive Costimulatory Molecules In Transplantation And Tolerance*," Curr. Opin. Organ Transplant. 13:366-372; Lenschow, D.J. *et al.* (1996) "*CD28/B7 System of T-Cell Costimulation*," Ann. Rev. Immunol. 14:233-258; Wang, S. *et al.* (2004) "*Co-Signaling Molecules Of The B7-CD28 Family In Positive And Negative Regulation Of T Lymphocyte Responses*," Microbes Infect. 6:759-766). There are

currently several known members of the family: B7.1 (CD80), B7.2 (CD86), the inducible co-stimulator ligand (ICOS-L), the programmed death-1 ligand (PD-L1; B7-H1), the programmed death-2 ligand (PD-L2; B7-DC), B7-H3, B7-H4 and B7-H6 (Collins, M. *et al.* (2005) "*The B7 Family Of Immune-Regulatory Ligands*," Genome Biol. 6:223.1-223.7; Flajnik, M.F. *et al.* (2012) "*Evolution Of The B7 Family: Co-Evolution Of B7H6 And Nkp30, Identification Of A New B7 Family Member, B7H7, And Of B7's Historical Relationship With The MHC*," Immunogenetics epub doi.org/10.1007/s00251-012-0616-2).

II. Programmed Death-1 ("PD-1")

[0008] Programmed Death-1 ("PD-1," also known as "CD279") is an approximately 31 kD type I membrane protein member of the extended CD28/CTLA-4 family of T-cell regulators that broadly negatively regulates immune responses (Ishida, Y. *et al.* (1992) "*Induced Expression Of PD-1, A Novel Member Of The Immunoglobulin Gene Superfamily, Upon Programmed Cell Death*," EMBO J. 11:3887-3895; United States Patent Application Publication No. 2007/0202100; 2008/0311117; 2009/00110667; United States Patents Nos. 6,808,710; 7,101,550; 7,488,802; 7,635,757; 7,722,868; PCT Publication No. WO 01/14557).

[0009] PD-1 is expressed on activated T-cells, B-cells, and monocytes (Agata, Y. *et al.* (1996) "*Expression Of The PD-1 Antigen On The Surface Of Stimulated Mouse T And B Lymphocytes*," Int. Immunol. 8(5):765-772; Yamazaki, T. *et al.* (2002) "*Expression Of Programmed Death 1 Ligands By Murine T-Cells And APC*," J. Immunol. 169:5538-5545) and at low levels in natural killer (NK) T-cells (Nishimura, H. *et al.* (2000) "*Facilitation Of Beta Selection And Modification Of Positive Selection In The Thymus Of PD-1-Deficient Mice*," J. Exp. Med. 191:891-898; Martin-Orozco, N. *et al.* (2007) "*Inhibitory Costimulation And Anti-Tumor Immunity*," Semin. Cancer Biol. 17(4):288-298).

[0010] The extracellular region of PD-1 consists of a single immunoglobulin (Ig)V domain with 23% identity to the equivalent domain in CTLA-4 (Martin-Orozco, N. *et al.* (2007) "*Inhibitory Costimulation And Anti-Tumor Immunity*," Semin. Cancer Biol. 17(4):288-298). The extracellular IgV domain is followed by a transmembrane region and an intracellular tail. The intracellular tail contains two phosphorylation sites located in an immunoreceptor tyrosine-based inhibitory motif and an immunoreceptor tyrosine-based switch motif, which suggests that PD-1 negatively regulates TCR signals (Ishida, Y. *et al.* (1992) "*Induced Expression Of PD-1, A Novel Member Of The Immunoglobulin Gene Superfamily, Upon Programmed Cell Death*," EMBO J. 11:3887-3895; Blank, C. *et al.* (2006) "*Contribution Of The PD-L1/PD-1*

Pathway To T-Cell Exhaustion: An Update On Implications For Chronic Infections And Tumor Evasion Cancer," Immunol. Immunother. 56(5):739-745).

[0011] PD-1 mediates its inhibition of the immune system by binding to B7-H1 and B7-DC (Flies, D.B. *et al.* (2007) "*The New B7s: Playing a Pivotal Role in Tumor Immunity*," J. Immunother. 30(3):251-260; United States Patents Nos. 6,803,192; 7,794,710; United States Patent Application Publication Nos. 2005/0059051; 2009/0055944; 2009/0274666; 2009/0313687; PCT Publication Nos. WO 01/39722; WO 02/086083).

[0012] B7-H1 and B7-DC are broadly expressed on the surfaces of human and murine tissues, such as heart, placenta, muscle, fetal liver, spleen, lymph nodes, and thymus as well as murine liver, lung, kidney, islets cells of the pancreas and small intestine (Martin-Orozco, N. *et al.* (2007) "*Inhibitory Costimulation And Anti-Tumor Immunity*," Semin. Cancer Biol. 17(4):288-298). In humans, B7-H1 protein expression has been found in human endothelial cells (Chen, Y. *et al.* (2005) "*Expression of B7-H1 in Inflammatory Renal Tubular Epithelial Cells*," Nephron. Exp. Nephrol. 102:e81-e92; de Haij, S. *et al.* (2005) "*Renal Tubular Epithelial Cells Modulate T-Cell Responses Via ICOS-L And B7-H1*" Kidney Int. 68:2091-2102; Mazanet, M.M. *et al.* (2002) "*B7-H1 Is Expressed By Human Endothelial Cells And Suppresses T-Cell Cytokine Synthesis*," J. Immunol. 169:3581-3588), myocardium (Brown, J.A. *et al.* (2003) "*Blockade Of Programmed Death-1 Ligands On Dendritic Cells Enhances T-Cell Activation And Cytokine Production*," J. Immunol. 170:1257-1266), syncyciotrophoblasts (Petroff, M.G. *et al.* (2002) "*B7 Family Molecules: Novel Immunomodulators At The Maternal-Fetal Interface*," Placenta 23:S95-S101). The molecules are also expressed by resident macrophages of some tissues, by macrophages that have been activated with interferon (IFN)-γ or tumor necrosis factor (TNF)-α (Latchman, Y. *et al.* (2001) "*PD-L2 Is A Second Ligand For PD-1 And Inhibits T-Cell Activation*," Nat. Immunol 2:261-268), and in tumors (Dong, H. (2003) "*B7-H1 Pathway And Its Role In The Evasion Of Tumor Immunity*," J. Mol. Med. 81:281-287).

[0013] The interaction between B7-H1 and PD-1 has been found to provide a crucial negative costimulatory signal to T and B-cells (Martin-Orozco, N. *et al.* (2007) "*Inhibitory Costimulation And Anti-Tumor Immunity*," Semin. Cancer Biol. 17(4):288-298) and functions as a cell death inducer (Ishida, Y. *et al.* (1992) "*Induced Expression Of PD-1, A Novel Member Of The Immunoglobulin Gene Superfamily, Upon Programmed Cell Death*," EMBO J. 11:3887-3895; Subudhi, S.K. *et al.* (2005) "*The Balance Of Immune Responses: Costimulation*

Verse Coinhibition," J. Molec. Med. 83:193-202). More specifically, interaction between low concentrations of the PD-1 receptor and the B7-H1 ligand has been found to result in the transmission of an inhibitory signal that strongly inhibits the proliferation of antigen-specific $CD8^+$ T-cells; at higher concentrations the interactions with PD-1 do not inhibit T-cell proliferation but markedly reduce the production of multiple cytokines (Sharpe, A.H. *et al.* (2002) "*The B7-CD28 Superfamily*," Nature Rev. Immunol. 2:116-126). T-cell proliferation and cytokine production by both resting and previously activated CD4 and CD8 T-cells, and even naive T-cells from umbilical-cord blood, have been found to be inhibited by soluble B7-H1-Fc fusion proteins (Freeman, G.J. *et al.* (2000) "*Engagement Of The PD-1 Immunoinhibitory Receptor By A Novel B7 Family Member Leads To Negative Regulation Of Lymphocyte Activation*," J. Exp. Med. 192:1-9; Latchman, Y. *et al.* (2001) "*PD-L2 Is A Second Ligand For PD-1 And Inhibits T-Cell Activation*," Nature Immunol. 2:261-268; Carter, L. *et al.* (2002) "*PD-1:PD-L Inhibitory Pathway Affects Both CD4(+) and CD8(+) T-cells And Is Overcome By IL-2*," Eur. J. Immunol. 32(3):634-643; Sharpe, A.H. *et al.* (2002) "*The B7-CD28 Superfamily*," Nature Rev. Immunol. 2:116-126).

[0014] The role of B7-H1 and PD-1 in inhibiting T-cell activation and proliferation has suggested that these biomolecules might serve as therapeutic targets for treatments of inflammation and cancer. Thus, the use of anti-PD-1 antibodies to treat infections and tumors and up-modulate an adaptive immune response has been proposed (see, United States Patent Application Publication Nos. 2010/0040614; 2010/0028330; 2004/0241745; 2008/0311117; 2009/0217401; United States Patents Nos. 7,521,051; 7,563,869; 7,595,048; PCT Publications Nos. WO 2004/056875; WO 2008/083174). Antibodies capable of specifically binding to PD-1 have been reported by Agata, T. *et al.* (1996) "*Expression Of The PD-1 Antigen On The Surface Of Stimulated Mouse T And B Lymphocytes*," Int. Immunol. 8(5):765-772; and Berger, R. *et al.* (2008) "*Phase I Safety And Pharmacokinetic Study Of CT-011, A Humanized Antibody Interacting With PD-1, In Patients With Advanced Hematologic Malignancies*," Clin. Cancer Res. 14(10):3044-3051 (see, also, United States Patent Nos. 8,008,449 and 8,552,154; US Patent Publication Nos. 2007/0166281; 2012/0114648; 2012/0114649; 2013/0017199; 2013/0230514 and 2014/0044738; and PCT Patent Publication Nos. WO 2003/099196; WO 2004/004771; WO 2004/056875; WO 2004/072286; WO 2006/121168; WO 2007/005874; WO 2008/083174; WO 2009/014708; WO 2009/073533; WO 2012/135408, WO 2012/145549; and WO 2013/014668).

[0015] However, despite all such prior advances, a need remains for improved compositions capable of more vigorously directing the body's immune system to attack cancer cells or pathogen-infected cells, especially at lower therapeutic concentrations. For although the adaptive immune system can be a potent defense mechanism against cancer and disease, it is often hampered by immune suppressive mechanisms in the tumor microenvironment, such as the expression of PD-1. Furthermore, co-inhibitory molecules expressed by tumor cells, immune cells, and stromal cells in the tumor milieu can dominantly attenuate T-cell responses against cancer cells. Thus, a need remains for potent PD-1-binding molecules. In particular, a need exists for potent PD-1-binding molecules having a desirable binding kinetic profile and that antagonize the PD-1/PD-L1 axis by blocking the PD-1/PD-L1 interaction, which could provide improved therapeutic value to patients suffering from cancer or other diseases and conditions. The present invention is directed to these and other goals.

Summary Of The Invention

[0016] The present invention is directed to PD-1 binding molecules that comprise the PD-1-binding domain of selected anti-PD-1 antibodies capable of binding to both cynomolgus monkey PD-1 and to human PD-1: PD-1 mAb 1, PD-1 mAb 2, PD-1 mAb 3, PD-1 mAb 4, PD-1 mAb 5, PD-1 mAb 6, PD-1 mAb 7, PD-1 mAb 8, PD-1 mAb 9, PD-1 mAb 10, PD-1 mAb 11, PD-1 mAb 12, PD-1 mAb 13, PD-1 mAb 14, or PD-1 mAb 15. The invention particularly concerns PD-1 binding molecules that are humanized or chimeric versions of such antibodies, or that comprise PD-1 binding-fragments of such anti-PD-1 antibodies (especially immunocongugates, diabodies, BiTEs, bispecific antibodies, *etc.*). The invention particularly concerns such PD-1-binding molecules that are additionally capable of binding an epitope of a molecule involved in regulating an immune check point that is present on the surface of an immune cell. The present invention also pertains to methods of using such PD-1-binding molecules to detect PD-1 or to stimulate an immune response. The present invention also pertains to methods of combination therapy in which a PD-1-binding molecule that comprises one or more PD-1-binding domain(s) of such selected anti-PD-1 antibodies is administered in combination with one or more additional molecules that are effective in stimulating an immune response and/or in combination with one or more additional molecules that specifically bind a cancer antigen.

[0017] In detail, the invention provides an anti-human PD-1-binding molecule that comprises the three Heavy Chain CDR Domains, CDR_H1, CDR_H2 and CDR_H3 and the three Light Chain CDR Domains, CDR_L1, CDR_L2, and CDR_L3, wherein:

(A) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of PD-1 mAb 1, and respectively have the amino acid sequences: **SEQ ID NO:71**, **SEQ ID NO:72**, and **SEQ ID NO:73**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of PD-1 mAb 1, and respectively have the amino acid sequences: **SEQ ID NO:76**, **SEQ ID NO:77**, and **SEQ ID NO:78**;

or

(B) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of PD-1 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:85**, **SEQ ID NO:86**, and **SEQ ID NO:87**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of PD-1 mAb 2, and, respectively have the amino acid sequences: **SEQ ID NO:90**, **SEQ ID NO:91**, and **SEQ ID NO:92**;

or

(C) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of PD-1 mAb 3, and respectively have the amino acid sequences: **SEQ ID NO:99**, **SEQ ID NO:100**, and **SEQ ID NO:101**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of PD-1 mAb 3, and, respectively have the amino acid sequences: **SEQ ID NO:104**, **SEQ ID NO:105**, and **SEQ ID NO:106**;

or

(D) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of PD-1 mAb 4, and respectively have the amino acid sequences: **SEQ ID NO:109**, **SEQ ID NO:110**, and **SEQ ID NO:111**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of PD-1 mAb 4, and, respectively have the amino acid sequences: **SEQ ID NO:114**, **SEQ ID NO:115**, and **SEQ ID NO:116**;

or

(E) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of PD-1 mAb 5, and respectively have the amino acid sequences: **SEQ ID NO:119**, **SEQ ID NO:120**, and **SEQ ID NO:121**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of PD-1 mAb 5, and, respectively have the amino acid sequences: **SEQ ID NO:124**, **SEQ ID NO:125**, and **SEQ ID NO:126**;

or

(F) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of PD-1 mAb 6, and respectively have the amino acid sequences: **SEQ ID NO:129**, **SEQ ID NO:130**, and **SEQ ID NO:131**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of PD-1 mAb 6, and, respectively have the amino acid sequences: **SEQ ID NO:134**, **SEQ ID NO:135**, and **SEQ ID NO:136**;

or

(G) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of PD-1 mAb 7, and respectively have the amino acid sequences: **SEQ ID NO:139**, **SEQ ID NO:140**, and **SEQ ID NO:141**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of PD-1 mAb 7, and, respectively have the amino acid sequences: **SEQ ID NO:144**, **SEQ ID NO:145**, and **SEQ ID NO:146**;

or

(H) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of PD-1 mAb 8, and respectively have the amino acid sequences: **SEQ ID NO:161**, **SEQ ID NO:162**, and **SEQ ID NO:163**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of PD-1 mAb 8, and, respectively have the amino acid sequences: **SEQ ID NO:166**, **SEQ ID NO:167**, and **SEQ ID NO:168**;

or

(I) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of PD-1 mAb 9, and respectively have the amino acid sequences: **SEQ ID NO:171**, **SEQ ID NO:172**, and **SEQ ID NO:173**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of PD-1 mAb 9, and, respectively have the amino acid sequences: **SEQ ID NO:176**, **SEQ ID NO:177**, and **SEQ ID NO:178**;

or

(J) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of PD-1 mAb 10, and respectively have the amino acid sequences: **SEQ ID NO:192**, **SEQ ID NO:193**, and **SEQ ID NO:194**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of PD-1 mAb 10, and, respectively have the amino acid sequences: **SEQ ID NO:197**, **SEQ ID NO:198**, and **SEQ ID NO:199**;

or

(K) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of PD-1 mAb 11, and respectively have the amino acid sequences: **SEQ ID NO:202**, **SEQ ID NO:203**, and **SEQ ID NO:204**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of PD-1 mAb 11, and, respectively have the amino acid sequences: **SEQ ID NO:207**, **SEQ ID NO:208**, and **SEQ ID NO:209**;

or

(L) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of PD-1 mAb 12, and respectively have the amino acid sequences: **SEQ ID NO:212**, **SEQ ID NO:213**, and **SEQ ID NO:214**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of PD-1 mAb 12, and, respectively have the amino acid sequences: **SEQ ID NO:217**, **SEQ ID NO:218**, and **SEQ ID NO:219**

or

(M) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of PD-1 mAb 13, and respectively have the amino

acid sequences: **SEQ ID NO:222**, **SEQ ID NO:223**, and **SEQ ID NO:224**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of PD-1 mAb 13, and, respectively have the amino acid sequences: **SEQ ID NO:227**, **SEQ ID NO:228**, and **SEQ ID NO:229**;

or

(N) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of PD-1 mAb 14, and respectively have the amino acid sequences: **SEQ ID NO:232**, **SEQ ID NO:233**, and **SEQ ID NO:234**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of PD-1 mAb 14, and, respectively have the amino acid sequences: **SEQ ID NO:237**, **SEQ ID NO:238**, and **SEQ ID NO:239**;

or

(O) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of PD-1 mAb 15, and respectively have the amino acid sequences: **SEQ ID NO:242**, **SEQ ID NO:243**, and **SEQ ID NO:244**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of PD-1 mAb 15, and, respectively have the amino acid sequences: **SEQ ID NO:247**, **SEQ ID NO:248**, and **SEQ ID NO:249**;

or

(P) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of hPD-1 mAb 7(1.2), and respectively have the amino acid sequences: **SEQ ID NO:139**, **SEQ ID NO:140**, and **SEQ ID NO:141**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of hPD-1 mAb 7(1.2), and, respectively have the amino acid sequences: **SEQ ID NO:157**, **SEQ ID NO:145**, and **SEQ ID NO:146**;

or

(Q) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of hPD-1 mAb 7(1.3), and respectively have the

amino acid sequences: **SEQ ID NO:139**, **SEQ ID NO:140**, and **SEQ ID NO:141**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of hPD-1 mAb 7(1.3), and, respectively have the amino acid sequences: **SEQ ID NO:157**, **SEQ ID NO:158**, and **SEQ ID NO:145**;

or

(R) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of hPD-1 mAb 9(2.2), and respectively have the amino acid sequences: **SEQ ID NO:183**, **SEQ ID NO:172**, and **SEQ ID NO:173**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of hPD-1 mAb 9(2.2), and, respectively have the amino acid sequences: **SEQ ID NO:188**, **SEQ ID NO:189**, and **SEQ ID NO:178**.

[0018] The invention further concerns the embodiments of all such anti-human PD-1-binding molecules wherein the molecule is an antibody, and especially wherein the molecule is a chimeric antibody or a humanized antibody.

[0019] The invention further concerns the embodiments of such anti-human PD-1-binding molecules wherein the Heavy Chain Variable Domain has the amino acid sequence of **SEQ ID NO:79**, **SEQ ID NO:93**, **SEQ ID NO:147**, **SEQ ID NO:149**, **SEQ ID NO:179**, **SEQ ID NO:181**, or **SEQ ID NO:250**.

[0020] The invention further concerns the embodiments of such anti-human PD-1-binding molecules wherein the Light Chain Variable Domain has the amino acid sequence of **SEQ ID NO:81**, **SEQ ID NO:95**, **SEQ ID NO:151**, **SEQ ID NO:153**, **SEQ ID NO:155**, **SEQ ID NO:184**, **SEQ ID NO:186**, or **SEQ ID NO:251**.

[0021] The invention further concerns the embodiment wherein the anti-human PD-1-binding molecule is a bispecific binding molecule, capable of simultaneously binding to human PD-1 and to a second epitope, and particularly concerns the embodiment wherein the second epitope is an epitope of a molecule involved in regulating an immune check point present on the surface of an immune cell (especially wherein the second epitope is an epitope of B7-H3,

B7-H4, BTLA, CD40, CD40L, CD47, CD70, CD80, CD86, CD94, CD137, CD137L, CD226, CTLA-4, Galectin-9, GITR, GITRL, HHLA2, ICOS, ICOSL, KIR, LAG-3, LIGHT, MHC class I or II, NKG2a, NKG2d, OX40, OX40L, PD1H, PD-1, PD-L1, PD-L2, PVR, SIRPa, TCR, TIGIT, TIM-3 or VISTA, and most particularly wherein the second epitope is an epitope of CD137, CTLA-4, LAG-3, OX40, TIGIT, or TIM-3).

[0022] The invention further concerns the embodiments wherein the anti-human PD-1-binding molecule is a bispecific molecule comprising a LAG-3 epitope-binding site, particularly wherein the LAG-3 epitope-binding site comprises:

(A) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain of the Variable Heavy Chain of LAG-3 mAb 1, having the amino acid sequences: **SEQ ID NO:42**, **SEQ ID NO:43**, and **SEQ ID NO:44**, respectively; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain of the Variable Light Chain of LAG-3 mAb 1, having the amino acid sequences: **SEQ ID NO:46**, **SEQ ID NO:47**, and **SEQ ID NO:48**, respectively;

or

(B) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain of the Variable Heavy Chain of hLAG-3 mAb 1 VH1, having the amino acid sequences: **SEQ ID NO:42**, **SEQ ID NO:43**, and **SEQ ID NO:44**, respectively; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain of the Variable Light Chain of hLAG-3 mAb 1 VL4, having the amino acid sequences: **SEQ ID NO:55**, **SEQ ID NO:47**, and **SEQ ID NO:48**, respectively;

or

(C) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain of the Variable Heavy Chain of LAG-3 mAb 6, having the amino acid sequences: **SEQ ID NO:57**, **SEQ ID NO:58**, and **SEQ ID NO:59**, respectively; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain of the Variable Light Chain of LAG-3 mAb 6, having the amino acid

sequences: **SEQ ID NO:61**, **SEQ ID NO:62**, and **SEQ ID NO:63**, respectively;

or

(D) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain of the Variable Heavy Chain of hLAG-3 mAb 6 VH1, having the amino acid sequences: **SEQ ID NO:57**, **SEQ ID NO:58**, and **SEQ ID NO:59**, respectively; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain of the Variable Light Chain of LAG-3 mAb 6, having the amino acid sequences: **SEQ ID NO:298**, **SEQ ID NO:62**, and **SEQ ID NO:63**, respectively.

[0023] The invention further concerns the embodiment of such anti-human PD-1-binding molecules wherein the molecule is a diabody, and especially, wherein the diabody is a covalently bonded complex that comprises two, or three, or four, or five polypeptide chains. The invention further concerns the embodiment of such anti-human PD-1-binding molecules wherein the molecule is a trivalent binding molecule, and especially wherein the trivalent binding molecule is a covalently bonded complex that comprises three, four, five or more than five polypeptide chains. The invention additionally concerns the embodiment of such anti-human PD-1-binding molecules in which the molecule comprises an Fc Region. The invention additionally concerns the embodiment of such anti-human PD-1-binding molecules in which the molecule comprises an Albumin-Binding Domain, and especially a deimmunized Albumin-Binding Domain.

[0024] The invention further concerns the embodiments of all such anti-human PD-1-binding molecules wherein the molecule comprises an Fc Region, and wherein the Fc Region is a variant Fc Region that comprises one or more amino acid modifications that reduces the affinity of the variant Fc Region for an FcγR and/or enhances the serum half-life, and more particularly, wherein the modifications comprise at least one amino acid substitution selected from the group consisting of:

(1) L234A; L235A;

(2) L234A and L235A;

(3) M252Y; M252Y and S254T;

(4) M252Y and T256E;

(5) M252Y, S254T and T256E; or

(6) K288D and H435K;

wherein the numbering is that of the EU index as in Kabat.

[0025] The invention further concerns the embodiments in which any of the above-described PD-1-binding molecules is used to stimulate a T-cell mediate immune response. The invention additionally concerns the embodiments in which any of the above-described PD-1-binding molecules is used in the treatment of a disease or condition associated with a suppressed immune system, especially cancer or an infection.

[0026] The invention particularly concerns such use in the treatment or diagnosis or prognosis of cancer, wherein the cancer is characterized by the presence of a cancer cell selected from the group consisting of a cell of: an adrenal gland tumor, an AIDS-associated cancer, an alveolar soft part sarcoma, an astrocytic tumor, bladder cancer, bone cancer, a brain and spinal cord cancer, a metastatic brain tumor, a breast cancer, a carotid body tumors, a cervical cancer, a chondrosarcoma, a chordoma, a chromophobe renal cell carcinoma, a clear cell carcinoma, a colon cancer, a colorectal cancer, a cutaneous benign fibrous histiocytoma, a desmoplastic small round cell tumor, an ependymoma, a Ewing's tumor, an extraskeletal myxoid chondrosarcoma, a fibrogenesis imperfecta ossium, a fibrous dysplasia of the bone, a gallbladder or bile duct cancer, gastric cancer, a gestational trophoblastic disease, a germ cell tumor, a head and neck cancer, hepatocellular carcinoma, an islet cell tumor, a Kaposi's Sarcoma, a kidney cancer, a leukemia, a lipoma/benign lipomatous tumor, a liposarcoma/malignant lipomatous tumor, a liver cancer, a lymphoma, a lung cancer, a medulloblastoma, a melanoma, a meningioma, a multiple endocrine neoplasia, a multiple myeloma, a myelodysplastic syndrome, a neuroblastoma, a neuroendocrine tumors, an ovarian cancer, a pancreatic cancer, a papillary thyroid carcinoma, a parathyroid tumor, a pediatric cancer, a peripheral nerve sheath tumor, a phaeochromocytoma, a pituitary tumor, a prostate cancer, a posterious uveal melanoma, a rare hematologic disorder, a renal metastatic cancer, a rhabdoid tumor, a rhabdomysarcoma, a sarcoma, a skin cancer, a soft-tissue sarcoma, a squamous cell cancer, a stomach cancer, a synovial sarcoma, a testicular cancer, a thymic carcinoma, a thymoma, a thyroid metastatic cancer, and a uterine cancer.

[0027] The invention particularly concerns such use in the treatment or diagnosis or prognosis of cancer, wherein the cancer is colorectal cancer, hepatocellular carcinoma, glioma, kidney cancer, breast cancer, multiple myeloma, bladder cancer, neuroblastoma; sarcoma, non-

Hodgkin's lymphoma, non-small cell lung cancer, ovarian cancer, pancreatic cancer, a rectal cancer, acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), acute B lymphoblastic leukemia (B-ALL), chronic lymphocytic leukemia (CLL), hairy cell leukemia (HCL), blastic plasmacytoid dendritic cell neoplasm (BPDCN), non-Hodgkin's lymphomas (NHL), including mantel cell leukemia (MCL), and small lymphocytic lymphoma (SLL), Hodgkin's lymphoma, systemic mastocytosis, or Burkitt's lymphoma.

[0028] The invention further concerns the embodiments in which any of the above-described PD-1-binding molecules is detectably labeled and is used in the detection of PD-1.

Brief Description of the Drawings

[0029] **Figure 1** provides a schematic of a representative covalently bonded diabody having two epitope-binding sites composed of two polypeptide chains, each having an E-coil or K-coil Heterodimer-Promoting Domain. A cysteine residue may be present in a linker and/or in the Heterodimer-Promoting Domain as shown in **Figure 3B**. VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern.

[0030] **Figure 2** provides a schematic of a representative covalently bonded diabody molecule having two epitope-binding sites composed of two polypeptide chains, each having a CH2 and CH3 Domain, such that the associated chains form all or part of an Fc Region. VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern.

[0031] **Figures 3A-3C** provide schematics showing representative tetravalent diabodies having four epitope-binding sites composed of two pairs of polypeptide chains (*i.e.*, four polypeptide chains in all). One polypeptide of each pair possesses a CH2 and CH3 Domain, such that the associated chains form all or part of an Fc Region. VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern. The two pairs of polypeptide chains may be same. In such embodiments wherein the VL and VH Domains recognize different epitopes (as shown in **Figures 3A-3C**), the resulting molecule possesses four epitope-binding sites and is bispecific and bivalent with respect to each bound epitope. In such embodiments wherein the VL and VH Domains recognize the same epitope (*e.g.*, the same VL Domain CDRs and the same VH Domain CDRs are used on both chains), the resulting molecule possesses four epitope-binding sites and is monospecific and tetravalent with respect to a single epitope. Alternatively, the two pairs of polypeptides may be different. In such

embodiments wherein the VL and VH Domains of each pair of polypeptides recognize different epitopes (as shown in **Figures 3A-3C**), the resulting molecule possesses four epitope-binding sites and is tetraspecific and monovalent with respect to each bound epitope. **Figure 3A** shows an Fc diabody which contains a peptide Heterodimer-Promoting Domain comprising a cysteine residue. **Figure 3B** shows an Fc Region-containing diabody, which contains E-coil and K-coil Heterodimer-Promoting Domains comprising a cysteine residue and a linker (with an optional cysteine residue). **Figure 3C**, shows an Fc-Region-Containing diabody, which contains antibody CH1 and CL domains.

[0032] **Figures 4A and 4B** provide schematics of a representative covalently bonded diabody molecule having two epitope-binding sites composed of three polypeptide chains. Two of the polypeptide chains possess a CH2 and CH3 Domain, such that the associated chains form all or part of an Fc Region. The polypeptide chains comprising the VL and VH Domain further comprise a Heterodimer-Promoting Domain. VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern.

[0033] **Figure 5** provides the schematics of a representative covalently bonded diabody molecule having four epitope-binding sites composed of five polypeptide chains. Two of the polypeptide chains possess a CH2 and CH3 Domain, such that the associated chains form an Fc Region that comprises all or part of an Fc Region. The polypeptide chains comprising the linked VL and VH Domains further comprise a Heterodimer-Promoting Domain. VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern.

[0034] **Figures 6A-6F** provide schematics of representative Fc Region-containing trivalent binding molecules having three epitope-binding sites. **Figures 6A** and **6B**, respectively, illustrate schematically the domains of trivalent binding molecules comprising two diabody-type binding domains and a Fab-type binding domain having different domain orientations in which the diabody-type binding domains are N-terminal or C-terminal to an Fc Region. The molecules in **Figures 6A** and **6B** comprise four chains. **Figures 6C** and **6D**, respectively, illustrate schematically the domains of trivalent binding molecules comprising two diabody-type binding domains N-terminal to an Fc Region, and a Fab-type binding domain in which the light chain and heavy chain are inked via a polypeptide spacer, or an scFv-type binding domain. The trivalent binding molecules in **Figures 6E** and **6F**, respectively illustrate schematically the domains of trivalent binding molecules comprising two diabody-type binding domains C-terminal to an Fc Region, and a linked Fab-type binding domain, or an scFv-type binding

domain in which the diabody-type binding domains are. The trivalent binding molecules in **Figures 6C-6F** comprise three chains. VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern.

[0035] **Figures 7A-7D** shows that the anti-PD-1 antibodies PD-1 mAb 1-15 bind to human PD-1. Binding curves for binding to shPD-1-His are shown in **Figure 7A** (PD-1 mAb 1, PD-1 mAb 2, PD-1 mAb 4 and PD-1 mAb 9), **Figure 7B** (PD-1 mAb 5, PD-1 mAb 6, and PD-1 mAb 7), and **Figure 7C** (PD-1 mAb 3, PD-1 mAb 8, PD-1 mAb 10, PD-1 mAb 11, PD-1 mAb 12, PD-1 mAb 13, PD-1 mAb 14, and PD-1 mAb 15). Binding curves for binding to shPD-1-human Fc are shown in **Figure 7D** (PD-1 mAb 3, PD-1 mAb 8, PD-1 mAb 10, PD-1 mAb 11, PD-1 mAb 12, PD-1 mAb 13, PD-1 mAb 14, and PD-1 mAb 15).

[0036] **Figures 8A-8C** shows that the anti-PD-1 antibodies PD-1 mAb 1-15 bind to cynomolgus monkey PD-1. Binding curves for binding to scynoPD-1-hFc are shown in **Figure 8A** (PD-1 mAb 1, PD-1 mAb 2, PD-1 mAb 4, PD-1 mAb 5, PD-1 mAb 6, PD-1 mAb 7), **Figure 8B** (PD-1 mAb 9), and **Figure 8C** (PD-1 mAb 3, PD-1 mAb 8, PD-1 mAb 10, PD-1 mAb 11, PD-1 mAb 12, PD-1 mAb 13, PD-1 mAb 14, and PD-1 mAb 15).

[0037] **Figures 9A-9D** show the ability of the anti-PD-1 antibodies PD-1 mAb 1-15 to block the binding of human PD-L1 to human PD-1. Inhibition curves are shown in **Figure 9A** (PD-1 mAb 1, PD-1 mAb 2, PD-1 mAb 3, PD-1 mAb 15, and PD-1 mAb A), **Figure 9B** (PD-1 mAb 4), **Figure 9C** (PD-1 mAb 5, PD-1 mAb 6, PD-1 mAb 7, and PD-1 mAb A), and **Figure 9D** (PD-1 mAb 3, PD-1 mAb 8, PD-1 mAb 10, PD-1 mAb 11, PD-1 mAb 12, PD-1 mAb 13, PD-1 mAb 14, PD-1 mAb 15, and PD-1 mAb A).

[0038] **Figures 10A-10B** show the tissue specificity of the anti-human PD-1 antibody PD-1 mAb 7. **Figure 10A** shows histological stains of normal colon (**Panels i** and **vii**), liver (**Panels ii** and **viii**), lung (**Panels iii** and **ix**), pancreas (**Panels iv** and **x**), kidney (**Panels v** and **xi**) and heart (**Panels vi** and **xii**) tissue. **Figure 10A, Panels i-vi** show the results of tissue incubated with labeled PD-1 mAb 7 (0.313 μg/mL). **Figure 10A, Panels vii-xii** show the results of tissue incubated with labeled isotype control mAb (0.314 μg/mL). **Figure 10B** shows histological stains of skin (**Panels i** and **iv**), tonsils (**Panels ii** and **v**), and NSO cells expressing PD-1 (**Panels iii** and **vi**). **Figure 10B, Panels i-iii** show the results of tissue incubated with labeled PD-1 mAb 7 (0.313 μg/mL).

[0039] **Figure 11** shows the binding profiles of humanized anti-human PD-1 antibodies hPD-1 mAb 2, hPD-1 mAb 7(1.1), hPD-1 mAb 7(1.2), hPD-1 mAb 9(1.1), and the reference anti-PD-1 antibodies PD-1 mAb A and PD-1 mAb B having IgG1 (AA) or IgG4 (P) for binding to cell surface PD-1.

[0040] **Figures 12A-12B** show the ability of humanized anti-PD antibodies hPD-1 mAb 2, hPD-1 mAb 7(1.1), hPD-1 mAb 7(1.2), hPD-1 mAb 9(1.1), and the reference anti-PD-1 antibodies PD-1 mAb A and PD-1 mAb B, having IgG1 (AA) or IgG4 (P) to block the binding of soluble human PD-L1 (**Figure 12A**) and soluble human PD-L2 (**Figure 12B**) to cell surface human PD-1.

[0041] **Figure 13** shows the ability of humanized anti-PD antibodies hPD-1 mAb 2, hPD-1 mAb 7(1.1), hPD-1 mAb 7(1.2), hPD-1 mAb 9(1.1), and the reference anti-PD-1 antibodies PD-1 mAb A and PD-1 mAb B, having IgG1 (AA) or IgG4 (P) to antagonize the PD-1/PD-L1 axis by blocking the PD-1/PD-L1 interaction and preventing down-regulation of T-cell responses in a Jurkat-luc-NFAT / CHO-PD-L1 luciferase reporter assay.

[0042] **Figure 14** shows that PD-1 mAb 2, PD-1 mAb 7, PD-1 mAb 9 and PD-1 mAb 15 are able to stimulate cytokine production to levels comparable or higher than the referenced anti-PD-1 antibodies (PD-1 mAb A and PD-1 mAb B) and that treatment with PD-1 mAb 2, PD-1 mAb 7, PD-1 mAb 9 and PD-1 mAb 15 in combination with LAG-3 mAb 1 provided the largest enhancement of cytokine release. IFNγ secretion profiles from *Staphylococcal* enterotoxin B (SEB)-stimulated PBMCs treated with anti-PD-1 and anti-LAG-3 antibodies alone and in combination.

[0043] **Figures 15A-15B** show the ability of humanized anti-PD antibodies hPD-1 mAb 2, hPD-1 mAb 7(1.2), hPD-1 mAb 9(1.1), and the reference anti-PD-1 antibodies PD-1 mAb A and PD-1 mAb B, having IgG1 (AA) or IgG4 (P) to stimulate cytokine production. IFNγ (**Figure 15A**) and TNFα (**Figure 15B**), secretion profiles from SEB-stimulated PBMCs treated with anti-PD-1 antibodies.

[0044] **Figures 16A-16B** show that the PD-1 x LAG-3 bispecific diabody constructs DART A, DART D, DART E, DART F, DART G and DART H, are able to stimulate cytokine production to levels comparable or higher than that observed upon the administration of the combination of an anti-PD-1 mAb + an anti-LAG-3 mAb (PD-1 mAb A + LAG-3 mAb A), and that the PD-1 x LAG-3 bispecific diabody constructs DART A, DART D, DART E, DART

F and DART G provided the largest enhancement of cytokine release. IFNγ secretion profiles of PBMCs stimulated with a low concentration of SEB (0.2 ng/mL treated with PD-1 x LAG-3 bispecific diabodies, or anti-PD-1 and anti-LAG-3 antibodies alone and in combination are plotted. The results using PBMCs from two representative donors are shown in **Figure 16A** and **Figure 16B**.

[0045] **Figures 17A-17B** show that the PD-1 x LAG-3 bispecific diabody constructs DART A, DART B and DART C are able to stimulate cytokine production to levels higher than that observed upon the administration of the combination of an anti-PD-1 mAb + an anti-LAG-3 mAb (PD-1 mAb A + LAG-3 mAb A). IFNγ secretion profiles of PBMCs from two representative donors, stimulated with a high concentration of SEB (85 ng/mL) treated with PD-1 x LAG-3 bispecific diabodies, or anti-PD-1 and anti-LAG-3 antibodies alone and in combination are plotted. The results using PBMCs from two representative donors are shown in **Figure 17A** and **Figure 17B**.

[0046] **Figures 18A-18B** show that the PD-1 x LAG-3 bispecific diabody constructs DART A, DART B and DART C are able to stimulate cytokine production to levels higher than that observed upon the administration of the combination of an anti-PD-1 mAb + an anti-LAG-3 mAb (PD-1 mAb A + LAG-3 mAb A). IFNγ secretion profiles of PBMCs from two representative donors, stimulated with a middle concentration of SEB (0.5 ng/mL) treated with PD-1 x LAG-3 bispecific diabodies, or anti-PD-1 and anti-LAG-3 antibodies alone and in combination are plotted. The results using PBMCs from two representative donors are shown in **Figure 18A** and **Figure 18B.**

[0047] **Figure 19** shows that the PD-1 x LAG-3 bispecific diabody constructs DART D and DART H are able to stimulate cytokine production to levels comparable or higher than that observed upon the administration of the combination of an anti-PD-1 mAb + an anti-LAG-3 mAb (PD-1 mAb A + LAG-3 mAb A), and that DART D provided the largest enhancement of cytokine release. IL-2 secretion profiles of PBMCs from a representative donor stimulated with a high concentration of SEB (85 ng/mL) treated with PD-1 x LAG-3 bispecific diabodies, or anti-PD-1 and anti-LAG-3 antibodies alone and in combination are plotted.

[0048] **Figure 20** shows that the PD-1 x LAG-3 bispecific diabody constructs DART B and DART I are able to stimulate cytokine production to levels higher than that observed upon the administration of the combination of an anti-PD-1 mAb + an anti-LAG-3 mAb (PD-1 mAb A

+ LAG-3 mAb A, hPD-1 mAb 7(1.2) + hLAG-3 mAb 1(1.4), hPD-1 mAb 7(1.2) + hLAG-3 mAb 6(1.1)). IFNγ secretion profiles of PBMCs from a representative donor, stimulated with a middle concentration of SEB (0.5 ng/mL) treated with PD-1 x LAG-3 bispecific diabodies, or anti-PD-1 and anti-LAG-3 antibodies alone and in combination are plotted.

[0049] **Figures 21A-21D** show that the that the PD-1 x LAG-3 bispecific diabody DART I is able to stimulate cytokine production to levels higher than that observed upon the administration of the combination of an anti-PD-1 mAb + an anti-LAG-3 mAb (PD-1 mAb A + LAG-3 mAb A). IFNγ (**Figures 21A and 21C**) and IL-2 (**Figures 21B and 21D**) secretion profiles of CD4 memory cells from two representative donors, stimulated with tetanus toxoid (5 μg/mL) treated with the PD-1 x LAG-3 bispecific diabody DART-I, anti-PD-1 and anti-LAG-3 antibodies in combination, or an isotype control are plotted. The results at day 7 using CD4 memory T cells from two representative donors are shown in **Figures 21A-B** and **Figures 21C-D**.

[0050] **Figure 22** shows that the the pharmacokinetics of the PD-1 x LAG-3 bispecific molecule, DART I are comparable to those of the anti-PD-1 antibody, PD-1 mAb A IgG4 (P) in cynomolgus monkey. The lines indicate the mean serum concentration of DART I (solid) and PD-1 mAb A (dashed). The individual values for the male (filled) and female (open) monkeys are plotted for DART I (triangles) and PD-1 mAb A (circles).

[0051] **Figures 23A-23C** show serum antibody concentrations and percentage of bound PD-1 on the surface of CD4+ or CD8+ T cells over time in animals following treatment with different anti-PD-1 antibodies. The percentage of bound PD 1 on the surface of CD4+ or CD8+ T cells following anti-PD 1 mAb treatment is plotted on the right y-axes; symbols represent % bound PD 1 on T cells for each individual animal and dashed lines represent the mean values. Serum mAb concentrations are plotted on the left y-axes; symbols represent serum levels for each individual animal and solid lines represent nonlinear fits of the data. Each panel presents data for animals (n = 1/sex/group) administered 10 mg/kg hPD-1 mAb 7 (1.2) IgG4 (P) (**Figure 23A**), PD-1 mAb A IgG4 (P) (**Figure 23B**), or PD-1 mAb B IgG4 (P) (**Figure 23B**) by IV infusion on Day 1.

Detailed Description of the Invention

[0052] The present invention is directed to PD-1-binding molecules that comprise the PD-1-binding domain of selectedanti-PD-1 antibodies capable of binding to both cynomolgus

monkey PD-1 and to human PD-1: PD-1 mAb 1, PD-1 mAb 2, PD-1 mAb 3, PD-1 mAb 4, PD-1 mAb 5, PD-1 mAb 6, PD-1 mAb 7, PD-1 mAb 8, PD-1 mAb 9, PD-1 mAb 10, PD-1 mAb 11, PD-1 mAb 12, PD-1 mAb 13, PD-1 mAb 14, or PD-1 mAb 15. The invention particularly concerns PD-1-binding molecules that are humanized or chimeric versions of such antibodies, or that comprise PD-1-binding fragments of such anti-PD-1 antibodies (especially immunocongugates, diabodies (including but not limited to DART-A, DART-B, DART-C, DART-D, DART-E, DART-F, DART-G, DART-H, DART-I, and DART-J), BiTEs, bispecific antibodies, *etc.*). The invention particularly concerns such PD-1-binding molecules that are additionally capable of binding an epitope of a molecule involved in regulating an immune check point that is present on the surface of an immune cell. The present invention also pertains to methods of using such PD-1-binding molecules to detect PD-1 or to stimulate an immune response. The present invention also pertains to methods of combination therapy in which a PD-1-binding molecule that comprises one or more PD-1-binding domain(s) of such selected anti-PD-1 antibodies is administered in combination with one or more additional molecules that are effective in stimulating an immune response and/or in combination with one or more additional molecules that specifically bind a cancer antigen.

I. Antibodies and Their Binding Domains

[0053] The antibodies of the present invention are immunoglobulin molecules capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid, polypeptide, *etc.*, through at least one antigen recognition site, located in the Variable Domain of the immunoglobulin molecule. As used herein, the terms "**antibody**" and "**antibodies**" refer to monoclonal antibodies, multispecific antibodies, human antibodies, humanized antibodies, synthetic antibodies, chimeric antibodies, polyclonal antibodies, camelized antibodies, single-chain Fvs (scFv), single-chain antibodies, Fab fragments, F(ab') fragments, disulfide-linked bispecific Fvs (sdFv), intrabodies, and epitope-binding fragments of any of the above. In particular, antibodies include immunoglobulin molecules and immunologically active fragments of immunoglobulin molecules, *i.e.*, molecules that contain an antigen-binding site. Immunoglobulin molecules can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA and IgY), class (*e.g.*, IgG_1, IgG_2, IgG_3, IgG_4, IgA_1 and IgA_2) or subclass. In addition to their known uses in diagnostics, antibodies have been shown to be useful as therapeutic agents. Antibodies are capable of immunospecifically binding to a polypeptide or protein or a non-protein molecule due to the presence on such molecule of a particular domain or moiety or conformation (an "**epitope**"). An epitope-containing molecule may have immunogenic activity, such that it

elicits an antibody production response in an animal; such molecules are termed "**antigens**"). The last few decades have seen a revival of interest in the therapeutic potential of antibodies, and antibodies have become one of the leading classes of biotechnology-derived drugs (Chan, C.E. *et al.* (2009) "*The Use Of Antibodies In The Treatment Of Infectious Diseases*," Singapore Med. J. 50(7):663-666). Over 200 antibody-based drugs have been approved for use or are under development.

[0054] The term "**monoclonal antibody**" refers to a homogeneous antibody population wherein the monoclonal antibody is comprised of amino acids (naturally occurring and non-naturally occurring) that are involved in the selective binding of an antigen. Monoclonal antibodies are highly specific, being directed against a single epitope (or antigenic site). The term "monoclonal antibody" encompasses not only intact monoclonal antibodies and full-length monoclonal antibodies, but also fragments thereof (such as Fab, Fab', $F(ab')_2$ Fv), single-chain (scFv), mutants thereof, fusion proteins comprising an antibody portion, humanized monoclonal antibodies, chimeric monoclonal antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site of the required specificity and the ability to bind to an antigen. It is not intended to be limited as regards to the source of the antibody or the manner in which it is made (*e.g.*, by hybridoma, phage selection, recombinant expression, transgenic animals, *etc.*). The term includes whole immunoglobulins as well as the fragments *etc.* described above under the definition of "antibody." Methods of making monoclonal antibodies are known in the art. One method which may be employed is the method of Kohler, G. *et al.* (1975) "*Continuous Cultures Of Fused Cells Secreting Antibody Of Predefined Specificity*," Nature 256:495-497 or a modification thereof. Typically, monoclonal antibodies are developed in mice, rats or rabbits. The antibodies are produced by immunizing an animal with an immunogenic amount of cells, cell extracts, or protein preparations that contain the desired epitope. The immunogen can be, but is not limited to, primary cells, cultured cell lines, cancerous cells, proteins, peptides, nucleic acids, or tissue. Cells used for immunization may be cultured for a period of time (*e.g.*, at least 24 hours) prior to their use as an immunogen. Cells may be used as immunogens by themselves or in combination with a non-denaturing adjuvant, such as Ribi (see, *e.g.*, Jennings, V.M. (1995) "*Review of Selected Adjuvants Used in Antibody Production*," ILAR J. 37(3):119-125). In general, cells should be kept intact and preferably viable when used as immunogens. Intact cells may allow antigens to be better detected than ruptured cells by the immunized animal. Use of denaturing or harsh adjuvants, *e.g.*, Freud's adjuvant, may rupture cells and

therefore is discouraged. The immunogen may be administered multiple times at periodic intervals such as, bi-weekly, or weekly, or may be administered in such a way as to maintain viability in the animal (*e.g.*, in a tissue recombinant). Alternatively, existing monoclonal antibodies and any other equivalent antibodies that are immunospecific for a desired pathogenic epitope can be sequenced and produced recombinantly by any means known in the art. In one embodiment, such an antibody is sequenced and the polynucleotide sequence is then cloned into a vector for expression or propagation. The sequence encoding the antibody of interest may be maintained in a vector in a host cell and the host cell can then be expanded and frozen for future use. The polynucleotide sequence of such antibodies may be used for genetic manipulation to generate the monospecific or multispecific (*e.g.*, bispecific, trispecific and tetraspecific) molecules of the invention as well as an affinity optimized, a chimeric antibody, a humanized antibody, and/or a caninized antibody, to improve the affinity, or other characteristics of the antibody. The general principle in humanizing an antibody involves retaining the basic sequence of the antigen-binding portion of the antibody, while swapping the non-human remainder of the antibody with human antibody sequences.

[0055] Natural antibodies (such as IgG antibodies) are composed of two **Light Chains** complexed with two **Heavy Chains**. Each light chain contains a Variable Domain (**VL**) and a Constant Domain (**CL**). Each heavy chain contains a Variable Domain (**VH**), three Constant Domains (**CH1**, **CH2** and **CH3**), and a hinge domain located between the **CH1** and **CH2** Domains. The basic structural unit of naturally occurring immunoglobulins (*e.g.*, IgG) is thus a tetramer having two light chains and two heavy chains, usually expressed as a glycoprotein of about 150,000 Da. The amino-terminal ("N-terminal") portion of each chain includes a Variable Domain of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal ("C-terminal") portion of each chain defines a constant region, with light chains having a single Constant Domain and heavy chains usually having three Constant Domains and a Hinge Domain. Thus, the structure of the light chains of an IgG molecule is n-VL-CL-c and the structure of the IgG heavy chains is n-VH-CH1-H-CH2-CH3-c (where H is the hinge domain, and n and c represent, respectively, the N-terminus and the C-terminus of the polypeptide). The Variable Domains of an IgG molecule consist of the complementarity determining regions (**CDR**), which contain the residues in contact with epitope, and non-CDR segments, referred to as framework segments (**FR**), which in general maintain the structure and determine the positioning of the CDR loops so as to permit such contacting (although certain framework residues may also contact antigen). Thus, the VL and

VH Domains have the structure n-FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4-c. Polypeptides that are (or may serve as) the first, second and third CDR of an antibody Light Chain are herein respectively designated **CDR_L1 Domain**, **CDR_L2 Domain**, and **CDR_L3 Domain**. Similarly, polypeptides that are (or may serve as) the first, second and third CDR of an antibody heavy chain are herein respectively designated **CDR_H1 Domain**, **CDR_H2 Domain**, and **CDR_H3 Domain**. Thus, the terms CDR_L1 Domain, CDR_L2 Domain, CDR_L3 Domain, CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are directed to polypeptides that when incorporated into a protein cause that protein to be able to bind to a specific epitope regardless of whether such protein is an antibody having light and heavy chains or a diabody or a single-chain binding molecule (*e.g.*, an scFv, a BiTe, *etc.*), or is another type of protein. Accordingly, as used herein, the term "**epitope-binding fragment**" means a fragment of an antibody capable of immunospecifically binding to an epitope, and the term "**epitope-binding site**" refers to that portion of a molecule comprising an epitope-binding fragment that is responsible for epitope binding. An epitope-binding site may contain 1, 2, 3, 4, 5 or all 6 of the CDR Domains of such antibody and, although capable of immunospecifically binding to such epitope, may exhibit an immunospecificity, affinity or selectivity toward such epitope that differs from that of such antibody. Preferably, however, an epitope-binding fragment will contain all 6 of the CDR Domains of such antibody. An epitope-binding fragment of an antibody may be a single polypeptide chain (*e.g.*, an scFv), or may comprise two or more polypeptide chains, each having an amino terminus and a carboxy terminus (*e.g.*, a diabody, a Fab fragment, an $F(ab')_2$ fragment, *etc.*).

[0056] The invention particularly encompasses single-chain Variable Domain fragments ("**scFv**") of the anti-PD-1 antibodies of this invention and multispecific binding molecules comprising the same. Single-chain Variable Domain fragments are made by linking Light and/or Heavy chain Variable Domain by using a short linking peptide. Bird *et al.* (1988) ("*Single-Chain Antigen-Binding Proteins*," Science 242:423-426) describes example of linking peptides which bridge approximately 3.5 nm between the carboxy terminus of one Variable Domain and the amino terminus of the other Variable Domain. Linkers of other sequences have been designed and used (Bird *et al.* (1988) "*Single-Chain Antigen-Binding Proteins*," Science 242:423-426). Linkers can in turn be modified for additional functions, such as attachment of drugs or attachment to solid supports. The single-chain variants can be produced either recombinantly or synthetically. For synthetic production of scFv, an automated synthesizer can be used. For recombinant production of scFv, a suitable plasmid containing polynucleotide

that encodes the scFv can be introduced into a suitable host cell, either eukaryotic, such as yeast, plant, insect or mammalian cells, or prokaryotic, such as E. coli. Polynucleotides encoding the scFv of interest can be made by routine manipulations such as ligation of polynucleotides. The resultant scFv can be isolated using standard protein purification techniques known in the art.

[0057] The invention also particularly encompasses humanized variants of the anti-PD-1 antibodies of the invention and multispecific binding molecules comprising the same. The term "**humanized**" antibody refers to a chimeric molecule, generally prepared using recombinant techniques, having an antigen-binding site of an immunoglobulin from a non-human species and a remaining immunoglobulin structure of the molecule that is based upon the structure and /or sequence of a human immunoglobulin. The anti-human PD-1 antibodies of the present invention include humanized, chimeric or caninized variants of antibodies PD-1 mAb 1, PD-1 mAb 2, PD-1 mAb 3, PD-1 mAb 4, PD-1 mAb 5, PD-1 mAb 6, PD-1 mAb 7, PD-1 mAb 8, PD-1 mAb 9, PD-1 mAb 10, PD-1 mAb 11, PD-1 mAb 12, PD-1 mAb 13, PD-1 mAb 14, or PD-1 mAb 15. The polynucleotide sequence of the variable domains of such antibodies may be used for genetic manipulation to generate such derivatives and to improve the affinity, or other characteristics of such antibodies. The general principle in humanizing an antibody involves retaining the basic sequence of the antigen-binding portion of the antibody, while swapping the non-human remainder of the antibody with human antibody sequences. There are four general steps to humanize a monoclonal antibody. These are: (1) determining the nucleotide and predicted amino acid sequence of the starting antibody light and heavy variable domains (2) designing the humanized antibody or caninized antibody, *i.e.*, deciding which antibody framework region to use during the humanizing or canonizing process (3) the actual humanizing or caninizing methodologies/techniques and (4) the transfection and expression of the humanized antibody. See, for example, U.S. Patents Nos. 4,816,567; 5,807,715; 5,866,692; and 6,331,415.

[0058] The antigen-binding site may comprise either a complete Variable Domain fused to a Constant Domain or only the complementarity determining regions (CDRs) of such Variable Domain grafted to appropriate framework regions. Antigen-binding sites may be wild-type or modified by one or more amino acid substitutions. This eliminates the constant region as an immunogen in human individuals, but the possibility of an immune response to the foreign variable domain remains (LoBuglio, A.F. *et al.* (1989) "*Mouse/Human Chimeric Monoclonal*

Antibody In Man: Kinetics And Immune Response," Proc. Natl. Acad. Sci. (U.S.A.) 86:4220-4224). Another approach focuses not only on providing human-derived constant regions, but modifying the variable domains as well so as to reshape them as closely as possible to human form. It is known that the variable domains of both heavy and light chains contain three complementarity determining regions (CDRs) which vary in response to the antigens in question and determine binding capability, flanked by four framework regions (FRs) which are relatively conserved in a given species and which putatively provide a scaffolding for the CDRs. When non-human antibodies are prepared with respect to a particular antigen, the variable domains can be "reshaped" or "humanized" by grafting CDRs derived from non-human antibody on the FRs present in the human antibody to be modified. Application of this approach to various antibodies has been reported by Sato, K. *et al.* (1993) Cancer Res 53:851-856. Riechmann, L. *et al.* (1988) "*Reshaping Human Antibodies for Therapy*," Nature 332:323-327; Verhoeyen, M. *et al.* (1988) "*Reshaping Human Antibodies: Grafting An Antilysozyme Activity*," Science 239:1534-1536; Kettleborough, C. A. *et al.* (1991) "*Humanization Of A Mouse Monoclonal Antibody By CDR-Grafting: The Importance Of Framework Residues On Loop Conformation*," Protein Engineering 4:773-3783; Maeda, H. *et al.* (1991) "*Construction Of Reshaped Human Antibodies With HIV-Neutralizing Activity*," Human Antibodies Hybridoma 2:124-134; Gorman, S. D. *et al.* (1991) "*Reshaping A Therapeutic CD4 Antibody*," Proc. Natl. Acad. Sci. (U.S.A.) 88:4181-4185; Tempest, P.R. *et al.* (1991) "*Reshaping A Human Monoclonal Antibody To Inhibit Human Respiratory Syncytial Virus Infection in vivo*," Bio/Technology 9:266-271; Co, M. S. *et al.* (1991) "*Humanized Antibodies For Antiviral Therapy*," Proc. Natl. Acad. Sci. (U.S.A.) 88:2869-2873; Carter, P. *et al.* (1992) "*Humanization Of An Anti-p185her2 Antibody For Human Cancer Therapy*," Proc. Natl. Acad. Sci. (U.S.A.) 89:4285-4289; and Co, M.S. *et al.* (1992) "*Chimeric And Humanized Antibodies With Specificity For The CD33 Antigen*," J. Immunol. 148:1149-1154. In some embodiments, humanized antibodies preserve all CDR sequences (for example, a humanized mouse antibody which contains all six CDRs from the mouse antibodies). In other embodiments, humanized antibodies have one or more CDRs (one, two, three, four, five, or six) which differ in sequence relative to the original antibody.

[0059] A number of "humanized" antibody molecules comprising an antigen-binding site derived from a non-human immunoglobulin have been described, including chimeric antibodies having rodent or modified rodent Variable Domain and their associated complementarity determining regions (CDRs) fused to human Constant Domains (see, for

example, Winter *et al.* (1991) "*Man-made Antibodies*," Nature 349:293-299; Lobuglio *et al.* (1989) "*Mouse/Human Chimeric Monoclonal Antibody In Man: Kinetics And Immune Response*," Proc. Natl. Acad. Sci. (U.S.A.) 86:4220-4224 (1989), Shaw *et al.* (1987) "*Characterization Of A Mouse/Human Chimeric Monoclonal Antibody (17-1A) To A Colon Cancer Tumor-Associated Antigen*," J. Immunol. 138:4534-4538, and Brown *et al.* (1987) "*Tumor-Specific Genetically Engineered Murine/Human Chimeric Monoclonal Antibody*," Cancer Res. 47:3577-3583). Other references describe rodent CDRs grafted into a human supporting framework region (FR) prior to fusion with an appropriate human antibody Constant Domain (see, for example, Riechmann, L. *et al.* (1988) "*Reshaping Human Antibodies for Therapy*," Nature 332:323-327; Verhoeyen, M. *et al.* (1988) "*Reshaping Human Antibodies: Grafting An Antilysozyme Activity*," Science 239:1534-1536; and Jones *et al.* (1986) "*Replacing The Complementarity-Determining Regions In A Human Antibody With Those From A Mouse*," Nature 321:522-525). Another reference describes rodent CDRs supported by recombinantly veneered rodent framework regions. See, for example, European Patent Publication No. 519,596. These "humanized" molecules are designed to minimize unwanted immunological response towards rodent anti-human antibody molecules, which limits the duration and effectiveness of therapeutic applications of those moieties in human recipients. Other methods of humanizing antibodies that may also be utilized are disclosed by Daugherty *et al.* (1991) "*Polymerase Chain Reaction Facilitates The Cloning, CDR-Grafting, And Rapid Expression Of A Murine Monoclonal Antibody Directed Against The CD18 Component Of Leukocyte Integrins*," Nucl. Acids Res. 19:2471-2476 and in U.S. Patents Nos. 6,180,377; 6,054,297; 5,997,867; and 5,866,692.

II. Fcγ Receptors (FcγRs)

[0060] The CH2 and CH3 Domains of the two heavy chains interact to form the **Fc Region**, which is a domain that is recognized by cellular **Fc Receptors**, including but not limited to Fc gamma Receptors **(FcγRs)**. As used herein, the term "Fc Region" is used to define a C-terminal region of an IgG heavy chain. The amino acid sequence of the CH2-CH3 Domain of an exemplary human IgG1 is (**SEQ ID NO:1**):

```
231    240         250         260         270         280
APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD

       290         300         310         320         330
GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
```

```
        340         350         360         370         380
PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE

        390         400         410         420         430
WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE

        440     447
ALHNHYTQKS LSLSPGX
```

as numbered by the EU index as set forth in Kabat, wherein, **X** is a lysine (K) or is absent.

[0061] The amino acid sequence of the CH2-CH3 Domain of an exemplary human IgG2 is (**SEQ ID NO:2**):

```
231     240         250         260         270         280
APPVA-GPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVQFNWYVD

        290         300         310         320         330
GVEVHNAKTK PREEQFNSTF RVVSVLTVVH QDWLNGKEYK CKVSNKGLPA

        340         350         360         370         380
PIEKTISKTK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDISVE

        390         400         410         420         430
WESNGQPENN YKTTPPMLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE

        440     447
ALHNHYTQKS LSLSPGX
```

as numbered by the EU index as set forth in Kabat, wherein, **X** is a lysine (K) or is absent.

[0062] The amino acid sequence of the CH2-CH3 Domain of an exemplary human IgG3 is (**SEQ ID NO:3**):

```
231     240         250         260         270         280
APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVQFKWYVD

        290         300         310         320         330
GVEVHNAKTK PREEQYNSTF RVVSVLTVLH QDWLNGKEYK CKVSNKALPA

        340         350         360         370         380
PIEKTISKTK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE

        390         400         410         420         430
WESSGQPENN YNTTPPMLDS DGSFFLYSKL TVDKSRWQQG NIFSCSVMHE

        440     447
ALHNRFTQKS LSLSPGX
```

as numbered by the EU index as set forth in Kabat, wherein, **X** is a lysine (K) or is absent.

[0063] The amino acid sequence of the CH2-CH3 Domain of an exemplary human IgG4 is (**SEQ ID NO:4**):

```
231     240         250         260         270         280
APEFLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSQED PEVQFNWYVD

        290         300         310         320         330
GVEVHNAKTK PREEQFNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKGLPS

        340         350         360         370         380
SIEKTISKAK GQPREPQVYT LPPSQEEMTK NQVSLTCLVK GFYPSDIAVE

        390         400         410         420         430
WESNGQPENN YKTTPPVLDS DGSFFLYSRL TVDKSRWQEG NVFSCSVMHE

        440     447
ALHNHYTQKS LSLSLGX
```

as numbered by the EU index as set forth in Kabat, wherein, **X** is a lysine (K) or is absent.

[0064] Throughout the present specification, the numbering of the residues in the constant region of an IgG heavy chain is that of the EU index as in Kabat *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, NH1, MD (1991) ("Kabat"), expressly incorporated herein by references. The term "EU index as in Kabat" refers to the numbering of the human IgG1 EU antibody. Amino acids from the Variable Domains of the mature heavy and light chains of immunoglobulins are designated by the position of an amino acid in the chain. Kabat described numerous amino acid sequences for antibodies, identified an amino acid consensus sequence for each subgroup, and assigned a residue number to each amino acid, and the CDRs are identified as defined by Kabat (it will be understood that CDR_H1 as defined by Chothia, C. & Lesk, A. M. ((1987) "*Canonical structures for the hypervariable regions of immunoglobulins*,". J. Mol. Biol. 196:901-917) begins five residues earlier). Kabat's numbering scheme is extendible to antibodies not included in his compendium by aligning the antibody in question with one of the consensus sequences in Kabat by reference to conserved amino acids. This method for assigning residue numbers has become standard in the field and readily identifies amino acids at equivalent positions in different antibodies, including chimeric or humanized variants. For example, an amino acid at position 50 of a human antibody light chain occupies the equivalent position to an amino acid at position 50 of a mouse antibody light chain.

[0065] Polymorphisms have been observed at a number of different positions within antibody constant regions (*e.g.*, CH1 positions, including but not limited to positions 192, 193, and 214; Fc positions, including but not limited to positions 270, 272, 312, 315, 356, and 358 as numbered by the EU index as set forth in Kabat), and thus slight differences between the presented sequence and sequences in the prior art can exist. Polymorphic forms of human immunoglobulins have been well-characterized. At present, 18 Gm allotypes are known: G1m (1, 2, 3, 17) or G1m (a, x, f, z), G2m (23) or G2m (n), G3m (5, 6, 10, 11, 13, 14, 15, 16, 21, 24, 26, 27, 28) or G3m (b1, c3, b3, b0, b3, b4, s, t, g1, c5, u, v, g5) (Lefranc, *et al.*, "*The Human IgG Subclasses: Molecular Analysis Of Structure, Function And Regulation.*" Pergamon, Oxford, pp. 43-78 (1990); Lefranc, G. *et al.*, 1979, Hum. Genet.: 50, 199-211). It is specifically contemplated that the antibodies of the present invention may be incorporate any allotype, isoallotype, or haplotype of any immunoglobulin gene, and are not limited to the allotype, isoallotype or haplotype of the sequences provided herein. Furthermore, in some expression systems the C-terminal amino acid residue (bolded above) of the CH3 Domain may be post-translationally removed. Accordingly, the C-terminal residue of the CH3 Domain is an optional amino acid residue in the PD-1-binding molecules of the invention. Specifically encompassed by the instant invention are PD-1-binding molecules lacking the C-terminal residue of the CH3 Domain. Also specifically encompassed by the instant invention are such constructs comprising the C-terminal lysine residue of the CH3 Domain.

[0066] Activating and inhibitory signals are transduced through the ligation of an Fc region to a cellular Fc gamma Receptor (FcγR). The ability of such ligation to result in diametrically opposing functions results from structural differences among the different FcγRs. Two distinct domains within the cytoplasmic signaling domains of the receptor called immunoreceptor tyrosine-based activation motifs (ITAMs) and immunoreceptor tyrosine-based inhibitory motifs (ITIMS) account for the different responses. The recruitment of different cytoplasmic enzymes to these structures dictates the outcome of the FcγR-mediated cellular responses. ITAM-containing FcγR complexes include FcγRI, FcγRIIA, FcγRIIIA, whereas ITIM-containing complexes only include FcγRIIB. Human neutrophils express the FcγRIIA gene. FcγRIIA clustering via immune complexes or specific antibody cross-linking serves to aggregate ITAMs along with receptor-associated kinases which facilitate ITAM phosphorylation. ITAM phosphorylation serves as a docking site for Syk kinase, activation of which results in activation of downstream substrates (*e.g.*, PI_3K). Cellular activation leads to release of proinflammatory mediators. The FcγRIIB gene is expressed on B lymphocytes; its

extracellular domain is 96% identical to FcγRIIA and binds IgG complexes in an indistinguishable manner. The presence of an ITIM in the cytoplasmic domain of FcγRIIB defines this inhibitory subclass of FcγR. Recently the molecular basis of this inhibition was established. When co-ligated along with an activating FcγR, the ITIM in FcγRIIB becomes phosphorylated and attracts the SH2 domain of the inositol polyphosphate 5'-phosphatase (SHIP), which hydrolyzes phosphoinositol messengers released as a consequence of ITAM-containing FcγR- mediated tyrosine kinase activation, consequently preventing the influx of intracellular Ca^{++}. Thus cross-linking of FcγRIIB dampens the activating response to FcγR ligation and inhibits cellular responsiveness. B-cell activation, B-cell proliferation and antibody secretion is thus aborted.

III. Bispecific Antibodies, Multispecific Diabodies and DART® Diabodies

[0067] The ability of an antibody to bind an epitope of an antigen depends upon the presence and amino acid sequence of the antibody's VL and VH Domains. Interaction of an antibody light chain and an antibody heavy chain and, in particular, interaction of its VL and VH Domains forms one of the two epitope-binding sites of a natural antibody. Natural antibodies are capable of binding to only one epitope species (*i.e.*, they are monospecific), although they can bind multiple copies of that species (*i.e.*, exhibiting bivalency or multivalency).

[0068] The binding domains of the present invention bind to epitopes in an "**immunospecific**" manner. As used herein, an antibody, diabody or other epitope-binding molecule is said to "**immunospecifically**" bind a region of another molecule (*i.e.*, an epitope) if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with that epitope relative to alternative epitopes. For example, an antibody that immunospecifically binds to a viral epitope is an antibody that binds this viral epitope with greater affinity, avidity, more readily, and/or with greater duration than it immunospecifically binds to other viral epitopes or non-viral epitopes. It is also understood by reading this definition that, for example, an antibody (or moiety or epitope) that immunospecifically binds to a first target may or may not specifically or preferentially bind to a second target. As such, "immunospecific binding" does not necessarily require (although it can include) exclusive binding. Generally, but not necessarily, reference to binding means "specific" binding. Two molecules are said to be capable of binding to one another in a "**physiospecific**" manner, if such binding exhibits the specificity with which receptors bind to their respective ligands.

[0069] The functionality of antibodies can be enhanced by generating multispecific antibody-based molecules that can simultaneously bind two separate and distinct antigens (or different epitopes of the same antigen) and/or by generating antibody-based molecule having higher valency (*i.e.*, more than two binding sites) for the same epitope and/or antigen.

[0070] In order to provide molecules having greater capability than natural antibodies, a wide variety of recombinant bispecific antibody formats have been developed (see, *e.g.*, PCT Publication Nos. WO 2008/003116, WO 2009/132876, WO 2008/003103, WO 2007/146968, WO 2009/018386, WO 2012/009544, WO 2013/070565), most of which use linker peptides either to fuse a further epitope-binding fragment (*e.g.*, an scFv, VL, VH, *etc.*) to, or within the antibody core (IgA, IgD, IgE, IgG or IgM), or to fuse multiple epitope-binding fragments (*e.g.*, two Fab fragments or scFvs). Alternative formats use linker peptides to fuse an epitope-binding fragment (*e.g.*, an scFv, VL, VH, *etc.*) to an a dimerization domain such as the CH2-CH3 Domain or alternative polypeptides (WO 2005/070966, WO 2006/107786A WO 2006/107617A, WO 2007/046893). Typically, such approaches involve compromises and trade-offs. For example, PCT Publications Nos. WO 2013/174873, WO 2011/133886 and WO 2010/136172 disclose that the use of linkers may cause problems in therapeutic settings, and teaches a trispecific antibody in which the CL and CH1 Domains are switched from their respective natural positions and the VL and VH Domains have been diversified (WO 2008/027236; WO 2010/108127) to allow them to bind to more than one antigen. Thus, the molecules disclosed in these documents trade binding specificity for the ability to bind additional antigen species. PCT Publications Nos. WO 2013/163427 and WO 2013/119903 disclose modifying the CH2 Domain to contain a fusion protein adduct comprising a binding domain. The document notes that the CH2 Domain likely plays only a minimal role in mediating effector function. PCT Publications Nos. WO 2010/028797, WO2010028796 and WO 2010/028795 disclose recombinant antibodies whose Fc Regions have been replaced with additional VL and VH Domains, so as to form trivalent binding molecules. PCT Publications Nos. WO 2003/025018 and WO2003012069 disclose recombinant diabodies whose individual chains contain scFv Domains. PCT Publications No. WO 2013/006544 discloses multivalent Fab molecules that are synthesized as a single polypeptide chain and then subjected to proteolysis to yield heterodimeric structures. Thus, the molecules disclosed in these documents trade all or some of the capability of mediating effector function for the ability to bind additional antigen species. PCT Publications Nos. WO 2014/022540, WO 2013/003652, WO 2012/162583, WO 2012/156430, WO 2011/086091, WO 2008/024188, WO 2007/024715,

WO 2007/075270, WO 1998/002463, WO 1992/022583 and WO 1991/003493 disclose adding additional binding domains or functional groups to an antibody or an antibody portion (*e.g.*, adding a diabody to the antibody's light chain, or adding additional VL and VH Domains to the antibody's light and heavy chains, or adding a heterologous fusion protein or chaining multiple Fab Domains to one another). Thus, the molecules disclosed in these documents trade native antibody structure for the ability to bind additional antigen species.

[0071] The art has additionally noted the capability to produce diabodies that differ from such natural antibodies in being capable of binding two or more different epitope species (*i.e.*, exhibiting bispecificity or multispecificity in addition to bivalency or multivalency) (see, *e.g.*, Holliger *et al.* (1993) "*'Diabodies': Small Bivalent And Bispecific Antibody Fragments,*" Proc. Natl. Acad. Sci. (U.S.A.) 90:6444-6448; US 2004/0058400 (Hollinger *et al.*); US 2004/0220388 / WO 02/02781 (Mertens *et al.*); Alt *et al.* (1999) FEBS Lett. 454(1-2):90-94; Lu, D. *et al.* (2005) "*A Fully Human Recombinant IgG-Like Bispecific Antibody To Both The Epidermal Growth Factor Receptor And The Insulin-Like Growth Factor Receptor For Enhanced Antitumor Activity*," J. Biol. Chem. 280(20):19665-19672; WO 02/02781 (Mertens *et al.*); Olafsen, T. *et al.* (2004) "*Covalent Disulfide-Linked Anti-CEA Diabody Allows Site-Specific Conjugation And Radiolabeling For Tumor Targeting Applications*," Protein Eng. Des. Sel. 17(1):21-27; Wu, A. *et al.* (2001) "*Multimerization Of A Chimeric Anti-CD20 Single Chain Fv-Fv Fusion Protein Is Mediated Through Variable Domain Exchange*," Protein Engineering 14(2):1025-1033; Asano *et al.* (2004) "*A Diabody For Cancer Immunotherapy And Its Functional Enhancement By Fusion Of Human Fc Domain*," Abstract 3P-683, J. Biochem. 76(8):992; Takemura, S. *et al.* (2000) "*Construction Of A Diabody (Small Recombinant Bispecific Antibody) Using A Refolding System,*" Protein Eng. 13(8):583-588; Baeuerle, P.A. *et al.* (2009) "*Bispecific T-Cell Engaging Antibodies For Cancer Therapy*," Cancer Res. 69(12):4941-4944).

[0072] The design of a diabody is based on the antibody derivative known as a single-chain Variable Domain fragment (**scFv**). Such molecules are made by linking Light and/ or Heavy chain Variable Domains by using a short linking peptide. Bird *et al.* (1988) ("*Single-Chain Antigen-Binding Proteins*," Science 242:423-426) describes example of linking peptides which bridge approximately 3.5 nm between the carboxy terminus of one Variable Domain and the amino terminus of the other Variable Domain. Linkers of other sequences have been designed and used (Bird *et al.* (1988) "*Single-Chain Antigen-Binding Proteins*," Science 242:423-426).

Linkers can in turn be modified for additional functions, such as attachment of drugs or attachment to solid supports. The single-chain variants can be produced either recombinantly or synthetically. For synthetic production of scFv, an automated synthesizer can be used. For recombinant production of scFv, a suitable plasmid containing polynucleotide that encodes the scFv can be introduced into a suitable host cell, either eukaryotic, such as yeast, plant, insect or mammalian cells, or prokaryotic, such as *E. coli*. Polynucleotides encoding the scFv of interest can be made by routine manipulations such as ligation of polynucleotides. The resultant scFv can be isolated using standard protein purification techniques known in the art.

[0073] The provision of non-monospecific diabodies provides a significant advantage over antibodies, including but not limited to, the capacity to co-ligate and co-localize cells that express different epitopes. Bispecific diabodies thus have wide-ranging applications including therapy and immunodiagnosis. Bispecificity allows for great flexibility in the design and engineering of the diabody in various applications, providing enhanced avidity to multimeric antigens, the cross-linking of differing antigens, and directed targeting to specific cell types relying on the presence of both target antigens. Due to their increased valency, low dissociation rates and rapid clearance from the circulation (for diabodies of small size, at or below ~50 kDa), diabody molecules known in the art have also shown particular use in the field of tumor imaging (Fitzgerald *et al.* (1997) *"Improved Tumour Targeting By Disulphide Stabilized Diabodies Expressed In Pichia pastoris,"* Protein Eng. 10:1221).

[0074] The bispecificity of diabodies has led to their use for co-ligating differing cells, for example, the cross-linking of cytotoxic T-cells to tumor cells (Staerz *et al.* (1985) *"Hybrid Antibodies Can Target Sites For Attack By T Cells,"* Nature 314:628-631, and Holliger *et al.* (1996) *"Specific Killing Of* Lymphoma *Cells By Cytotoxic T-Cells Mediated By A Bispecific Diabody,"* Protein Eng. 9:299-305; Marvin *et al.* (2005) "*Recombinant Approaches To IgG-Like Bispecific Antibodies*," Acta Pharmacol. Sin. 26:649-658). Alternatively, or additionally, bispecific diabodies can be used to co-ligate receptors on the surface of different cells or on a single cell. Co-ligation of different cells and/or receptors is useful to modulation effector functions and/or immune cell signaling. Multispecific molecules (*e.g.*, bispecific diabodies) comprising epitope-binding sites may be directed to a surface determinant of any immune cell such as B7-H3 (CD276), B7-H4 (VTCN1), BTLA (CD272), CD3, CD8, CD16, CD27, CD32, CD40, CD40L, CD47, CD64, CD70 (CD27L), CD80 (B7-1), CD86 (B7-2), CD94 (KLRD1), CD137 (4-1BB), CD137L (4-1BBL), CD226, CTLA-4 (CD152), Galectin-9, GITR, GITRL,

HHLA2, ICOS (CD278), ICOSL (CD275), Killer Activation Receptor (KIR), LAG-3 (CD223), LIGHT (TNFSF14, CD258), MHC class I or II, NKG2a, NKG2d, OX40 (CD134), OX40L (CD134L), PD1H, PD-1 (CD279), PD-L1 (B7-H1, CD274), PD-L2 (B7-CD, CD273), PVR (NECL5, CD155), SIRPa, TCR, TIGIT, TIM-3 (HAVCR2), and/or VISTA (PD-1H), which are expressed on T lymphocytes, Natural Killer (NK) cells, Antigen-presenting cells or other mononuclear cell. In particular, epitope-binding sites directed to a cell surface receptor that is involved in regulating an immune checkpoint (or the ligand thereof) are useful in the generation of bispecific or multispecific binding molecules which antagonize or block the inhibitory signaling of immune checkpoint molecules and thereby stimulate, upregulate or enhance, immune responses in a subject. Molecules involved in regulating immune checkpoints include, but are not limited to B7-H3, B7-H4, BTLA, CD40, CD40L, CD47, CD70, CD80, CD86, CD94, CD137, CD137L, CD226, CTLA-4, Galectin-9, GITR, GITRL, HHLA2, ICOS, ICOSL, KIR, LAG-3, LIGHT, MHC class I or II, NKG2a, NKG2d, OX40, OX40L, PD1H, PD-1, PD-L1, PD-L2, PVR, SIRPa, TCR, TIGIT, TIM-3 and/or VISTA.

[0075] However, the above advantages come at a salient cost. The formation of such non-monospecific diabodies requires the successful assembly of two or more distinct and different polypeptides (*i.e.*, such formation requires that the diabodies be formed through the heterodimerization of different polypeptide chain species). This fact is in contrast to monospecific diabodies, which are formed through the homodimerization of identical polypeptide chains. Because at least two dissimilar polypeptides (*i.e.*, two polypeptide species) must be provided in order to form a non-monospecific diabody, and because homodimerization of such polypeptides leads to inactive molecules (Takemura, S. *et al.* (2000) "*Construction Of A Diabody (Small Recombinant Bispecific Antibody) Using A Refolding System,*" Protein Eng. 13(8):583-588), the production of such polypeptides must be accomplished in such a way as to prevent covalent bonding between polypeptides of the same species (*i.e.*, so as to prevent homodimerization) (Takemura, S. *et al.* (2000) "*Construction Of A Diabody (Small Recombinant Bispecific Antibody) Using A Refolding System,*" Protein Eng. 13(8):583-588). The art has therefore taught the non-covalent association of such polypeptides (see, *e.g.*, Olafsen *et al.* (2004) *"Covalent Disulfide-Linked Anti-CEA Diabody Allows Site-Specific Conjugation And Radiolabeling For Tumor Targeting Applications,"* Prot. Engr. Des. Sel. 17:21-27; Asano *et al.* (2004) "*A Diabody For Cancer Immunotherapy And Its Functional Enhancement By Fusion Of Human Fc Domain,*" Abstract 3P-683, J. Biochem. 76(8):992; Takemura, S. *et al.* (2000) "*Construction Of A Diabody (Small Recombinant Bispecific*

Antibody) Using A Refolding System," Protein Eng. 13(8):583-588; Lu, D. *et al.* (2005) "*A Fully Human Recombinant IgG-Like Bispecific Antibody To Both The Epidermal Growth Factor Receptor And The Insulin-Like Growth Factor Receptor For Enhanced Antitumor Activity*," J. Biol. Chem. 280(20):19665-19672).

[0076] However, the art has recognized that bispecific diabodies composed of non-covalently associated polypeptides are unstable and readily dissociate into non-functional monomers (see, *e.g.*, Lu, D. *et al.* (2005) "*A Fully Human* Recombinant *IgG-Like Bispecific Antibody To Both The Epidermal Growth Factor Receptor And The Insulin-Like Growth Factor Receptor For Enhanced Antitumor Activity*," J. Biol. Chem. 280(20):19665-19672).

[0077] In the face of this challenge, the art has succeeded in developing stable, covalently bonded heterodimeric non-monospecific diabodies, termed **DART® (Dual Affinity Re-Targeting Reagents)** diabodies; see, *e.g.*, United States Patent Publications No. 2013-0295121; 2010-0174053 and 2009-0060910; European Patent Publication No. EP 2714079; EP 2601216; EP 2376109; EP 2158221 and PCT Publications No. WO 2012/162068; WO 2012/018687; WO 2010/080538; and Sloan, D.D. *et al.* (2015) "*Targeting HIV Reservoir in Infected CD4 T Cells by Dual-Affinity Re-targeting Molecules (DARTs) that Bind HIV Envelope and Recruit Cytotoxic T Cells*," PLoS Pathog. 11(11):e1005233. doi: 10.1371/journal.ppat.1005233; Al Hussaini, M. *et al.* (2015) "*Targeting CD123 In AML Using A T-Cell Directed Dual-Affinity Re-Targeting (DART®) Platform*," Blood pii: blood-2014-05-575704; Chichili, G.R. *et al.* (2015) "*A CD3xCD123 Bispecific DART For Redirecting Host T Cells To Myelogenous Leukemia: Preclinical Activity And Safety In Nonhuman Primates*," Sci. Transl. Med. 7(289):289ra82; Moore, P.A. *et al.* (2011) "*Application Of Dual Affinity Retargeting Molecules To Achieve Optimal Redirected T-Cell Killing Of B-Cell Lymphoma*," Blood 117(17):4542-4551; Veri, M.C. *et al.* (2010) "*Therapeutic Control Of B Cell Activation Via Recruitment Of Fcgamma Receptor IIb (CD32B) Inhibitory Function With A Novel Bispecific Antibody Scaffold*," Arthritis Rheum. 62(7):1933-1943; Johnson, S. *et al.* (2010) "*Effector Cell Recruitment With Novel Fv-Based Dual-Affinity Re-Targeting Protein Leads To Potent Tumor Cytolysis And in vivo B-Cell Depletion*," J. Mol. Biol. 399(3):436-449). Such diabodies comprise two or more covalently complexed polypeptides and involve engineering one or more cysteine residues into each of the employed polypeptide species that permit disulfide bonds to form and thereby covalently bond two polypeptide chains. For example, the addition of a cysteine residue to the C-terminus of such constructs has been shown to allow

disulfide bonding between the polypeptide chains, stabilizing the resulting heterodimer without interfering with the binding characteristics of the bivalent molecule.

[0078] Each of the two polypeptides of the simplest bispecific **DART®** diabody comprises three domains. The first polypeptide comprises (in the N-terminal to C-terminal direction): (i) a First Domain that comprises a binding region of a Light Chain Variable Domain of a first immunoglobulin (VL1), (ii) a Second Domain that comprises a binding region of a Heavy Chain Variable Domain of a second immunoglobulin (VH2), and (iii) a Third Domain that contains a cysteine residue (or a cysteine-containing domain) and a Heterodimer-Promoting Domain that serves to promote heterodimerization with the second polypeptide of the diabody and to covalently bond the diabody's first and second polypeptides to one another. The second polypeptide contains (in the N-terminal to C-terminal direction): (i) a First Domain that comprises a binding region of a Light Chain Variable Domain of the second immunoglobulin (VL2), (ii) a Second Domain that comprises a binding region of a Heavy Chain Variable Domain of the first immunoglobulin (VH1), and (iii) a Third Domain that contains a cysteine residue (or a cysteine-containing domain) and a complementary Heterodimer-Promoting Domain that complexes with the Heterodimer-Promoting Domain of the first polypeptide chain in order to promote heterodimerization with the first polypeptide chain. The cysteine residue (or a cysteine-containing domain) of the third domain of the second polypeptide chain serves to promote the covalent bonding of the second polypeptide chain to the first polypeptide chain of the diabody. Such molecules are stable, potent and have the ability to simultaneously bind two or more antigens. In one embodiment, the Third Domains of the first and second polypeptides each contain a cysteine residue, which serves to bind the polypeptides together via a disulfide bond. **Figure 1** provides a schematic of such a diabody, which utilizes E-coil/K-coil Heterodimer-Promoting domains and a cysteine containing linker for covalent bonding. As provided in **Figure 2** and **Figures 3A-3C**, one or both of the polypeptides may additionally possesses the sequence of a CH2-CH3 Domain, such that complexing between the two diabody polypeptides forms an Fc Region that is capable of binding to the Fc receptor of cells (such as B lymphocytes, dendritic cells, natural killer cells, macrophages, neutrophils, eosinophils, basophils and mast cells). As provided in more detail below, the CH2 and/or CH3 Domains of such polypeptide chains need not be identical in sequence, and advantageously are modified to foster complexing between the two polypeptide chains.

[0079] Many variations of such molecules have been described (see, *e.g.*, United States Patent Publications No. 2015/0175697; 2014/0255407; 2014/0099318; 2013/0295121; 2010/0174053 and 2009/0060910; European Patent Publication No. EP 2714079; EP 2601216; EP 2376109; EP 2158221 and PCT Publications No. WO 2012/162068; WO 2012/018687; WO 2010/080538). These Fc Region-containing **DART®** diabodies may comprise two pairs of polypeptide chains. The first polypeptide chain comprises (in the N-terminal to C-terminal direction): (i) a First Domain that comprises a binding region of a Light Chain Variable Domain of a first immunoglobulin (VL1), (ii) a Second Domain that comprises a binding region of a Heavy Chain Variable Domain of a second immunoglobulin (VH2), (iii) a Third Domain that contains a cysteine residue (or a cysteine-containing domain) and a serves to promote heterodimerization with the second polypeptide of the diabody and to covalently bond the diabody's first and second polypeptides to one another, and (iv) a CH2-CH3 Domain. The second polypeptide contains (in the N-terminal to C-terminal direction): (i) a First Domain that comprises a binding region of a Light Chain Variable Domain of the second immunoglobulin (VL2), (ii) a Second Domain that comprises a binding region of a Heavy Chain Variable Domain of the first immunoglobulin (VH1), and (iii)) a Third Domain that contains a cysteine residue (or a cysteine-containing domain) and a Heterodimer-Promoting Domain that promotes heterodimerization with the first polypeptide chain. Here two first polypeptides complex with each other to form an Fc Region. **Figures 3A-3C** provide schematics of three variations of such diabodies utilizing different Heterodimer-Promoting Domains.

[0080] Other Fc-Region-containing DART® diabodies may comprise three polypeptide chains. The first polypeptide of such DART® diabodies contains three domains: (i) a VL1-containing Domain, (ii) a VH2-containing Domain and (iii) a Domain containing a CH2-CH3 sequence. The second polypeptide of such DART® diabodies contains: (i) a VL2-containing Domain, (ii) a VH1-containing Domain and (iii) a Domain that promotes heterodimerization and covalent bonding with the diabody's first polypeptide chain. The third polypeptide of such DART® diabodies comprises a CH2-CH3 sequence. Thus, the first and second polypeptide chains of such DART® diabodies associate together to form a VL1/VH1 binding site that is capable of binding to the epitope, as well as a VL2/VH2 binding site that is capable of binding to the second epitope. Such more complex DART® molecules also possess cysteine-containing domains which function to form a covalently bonded complex. Thus, the first and second polypeptides are bonded to one another through a disulfide bond involving cysteine residues in their respective Third Domains. Notably, the first and third polypeptide chains

complex with one another to form an Fc Region that is stabilized via a disulfide bond. **Figures 4A-4B** provide schematics of such diabodies comprising three polypeptide chains.

[0081] Still other Fc-Region-containing DART® diabodies may comprise five polypeptide chains which may comprise the binding regions from the Light and Heavy Chain Variable Domains of up to three different immunoglobulins (referred to as VL1/VH1, VL2/VH2 and VL3/VH3). For example, the first polypeptide chain of such diabodies may contain: (i) a VH1-containing domain, (ii) a CH1-containing domain, and (iii) a Domain containing a CH2-CH3 sequence. The second and fifth polypeptide chains of such diabodies may contain: (i) a VL1-containing domain, and (ii) a CL-containing domain. The third polypeptide chain of such diabodies may contain: (i) a VH1-containing domain, (ii) a CH1-containing domain, (iii) a Domain containing a CH2-CH3 sequence, (iv) a VL2-containing Domain, (v) a VH3-containing Domain and (vi) a Heterodimer-Promoting Domain, where the Heterodimer-Promoting Domains promote the dimerization of the third chain with the fourth chain. The fourth polypeptide of such diabodies may contain: (i) a VL3-containing Domain, (ii) a VH2-containing Domain and (iii) a Domain that promotes heterodimerization and covalent bonding with the diabody's third polypeptide chain. Here the first and third polypeptides complex with each other to form an Fc Region. Such more complex DART® molecules also possess cysteine-containing domains which function to form a covalently bonded complex, such that each polypeptide chain is bonded to at least one addition polypeptide chain through a disulfide bond involving cysteine residues. Preferably, such domains are ordered in the N-terminal to C-terminal direction. **Figure 5** provides schematics of such diabodies comprising five polypeptide chains.

[0082] Alternative constructs are known in the art for applications where a tetravalent molecule is desirable but an Fc is not required including, but not limited to, tetravalent tandem antibodies, also referred to as "**TandAbs**" (see, *e.g.* United States Patent Publications Nos. 2005-0079170, 2007-0031436, 2010-0099853, 2011-020667 2013-0189263; European Patent Publication Nos. EP 1078004, EP 2371866, EP 2361936 and EP 1293514; PCT Publications Nos. WO 1999/057150, WO 2003/025018, and WO 2013/013700) which are formed by the homo-dimerization of two identical chains each possessing a VH1, VL2, VH2, and VL2 Domain.

[0083] Recently, trivalent structures incorporating two diabody-type binding domains and one non-diabody-type domain and an Fc Region have been described (see, *e.g.*, PCT

Application No: PCT/US15/33076, titled "Tri-Specific Binding Molecules and Methods of Use Thereof," filed May 29, 2015; and PCT/US15/33081, titled "Tri-Specific Binding Molecules That Specifically Bind to Multiple Cancer Antigens and Methods of Use Thereof," filed May 29, 2015). Such trivalent molecules may be utilized to generate monospecific, bispecific or trispecific molecules. **Figures 6A-6F** provide schematics of such trivalent molecules comprising 3 or 4 polypeptide chains.

IV. The Anti-Human PD-1-Binding Molecules of the Present Invention

[0084] The preferred PD-1-binding molecules of the present invention include antibodies, diabodies, BiTEs, *etc.* and are capable of binding to a continuous or discontinuous (*e.g.*, conformational) portion (**epitope**) of human PD-1 (CD279). The PD-1-binding molecules of the present invention will preferably also exhibit the ability to bind to PD-1 molecules of one or more non-human species, in particular, primate species (and especially a primate species, such as cynomolgus monkey). A representative human PD-1 polypeptide (NCBI Sequence NP_005009.2; including a 20 amino acid residue signal sequence (shown underlined) and the 268 amino acid residue mature protein) has the amino acid sequence (**SEQ ID NO:68**):

MQIPQAPWPV VWAVLQLGWR PGWFLDSPDR PWNPPTFSPA LLVVTEGDNA
TFTCSFSNTS ESFVLNWYRM SPSNQTDKLA AFPEDRSQPG QDCRFRVTQL
PNGRDFHMSV VRARRNDSGT YLCGAISLAP KAQIKESLRA ELRVTERRAE
VPTAHPSPSP RPAGQFQTLV VGVVGGLLGS LVLLVWVLAV ICSRAARGTI
GARRTGQPLK EDPSAVPVFS VDYGELDFQW REKTPEPPVP CVPEQTEYAT
IVFPSGMGTS SPARRGSADG PRSAQPLRPE DGHCSWPL

[0085] In certain embodiments the anti-human PD-1-binding molecules of the invention are characterized by any (one or more) of the following criteria:

(1) specifically binds human PD-1 as endogenously expressed on the surface of a stimulated human T-cell;

(2) specifically binds human PD-1 with an equilibrium binding constant (K_D) of 40 nM or less;

(3) specifically binds human PD-1 with an equilibrium binding constant (K_D) of 5 nM or less;

(4) specifically binds human PD-1 with an on rate (k_a) of 1.5×10^4 $M^{-1}min^{-1}$ or more;

(5) specifically binds human PD-1 with an on rate (k_a) of 90.0×10^4 $M^{-1}min^{-1}$ or more;

(6) specifically binds human PD-1 with an off rate (k_d) of 7×10^{-4} min^{-1} or less;

(7) specifically binds human PD-1 with an off rate (k_d) of 2 x 10^{-4} min^{-1} or less;

(8) specifically binds non-human primate PD-1 (*e.g.*, PD-1 of cynomolgus monkey);

(9) inhibits (*i.e.*, blocks or interferes with) the binding/the inhibitory activity) of PD-1 ligand (PD-L1/PD-L2) to PD-1;

(10) stimulates an immune response; and/or

(11) synergizes with an anti-human LAG-3 antibody to stimulate an antigen specific T-cell response.

[0086] As used here the term "antigen specific T-cell response" refers to responses by a T-cell that result from stimulation of the T-cell with the antigen for which the T-cell is specific. Non-limiting examples of responses by a T-cell upon antigen specific stimulation include proliferation and cytokine production (*e.g.*, TNF-α, IFN-γ production). The ability of a molecule to stimulate an antigen specific T-cell response may be determined, for example, using the *Staphylococcus aureus* Enterotoxin type B antigen ("**SEB**")-stimulated PBMC assay described herein.

[0087] The preferred anti-human PD-1-binding molecules of the present invention possess the VH and/or VL Domains of murine anti-human PD-1 monoclonal antibodies "**PD-1 mAb 1**," "**PD-1 mAb 2**," "**PD-1 mAb 3**,""**PD-1 mAb 4**," "**PD-1 mAb 5**," "**PD-1 mAb** 6," "**PD-1 mAb 7**," "**PD-1 mAb 8**," "**PD-1 mAb 9**," "**PD-1 mAb 10**," "**PD-1 mAb 11**," "**PD-1 mAb 12**," "**PD-1 mAb 13**," "**PD-1 mAb 14**," or "**PD-1 mAb 15**," and more preferably possess 1, 2 or all 3 of the CDR_{H}s of the VH Domain and/or 1, 2 or all 3 of the CDR_{L}s of the VL Domain of such anti-human PD-1 monoclonal antibodies. Such preferred anti-human PD-1-binding molecules include bispecific (or multispecific) antibodies, chimeric or humanized antibodies, BiTes, diabodies, *etc*, and such binding molecules having variant Fc Regions.

[0088] The invention particularly relates to PD-1-binding molecules comprising a PD-1 binding domain that possess:

(A) (1) the three CDR_{H}s of the VH Domain of PD-1mAb 1;

(2) the three CDR_{L}s of the VL Domain of PD-1 mAb 1;

(3) the three CDR_{H}s of the VH Domain of PD-1 mAb 1 and the three CDR_{L}s of the VL Domain of PD-1 mAb 1;

(4) the VH Domain of hPD-1 mAb 1 VH1;

(5) the VL Domain of hPD-1 mAb 1 VL1;

(6) the VH and VL Domains of hPD-1 mAb 1;

(B) (1) the three CDR_{HS} of the VH Domain of PD-1 mAb 2;

(2) the three CDR_{LS} of the VL Domain of the PD-1 mAb 2;

(3) the three CDR_{HS} of the VH Domain of PD-1 mAb 2 and the three CDR_{LS} of the VL Domain of PD-1 mAb 2;

(4) the VH Domain of hPD-1 mAb 2 VH1;

(5) the VL Domain of hPD-1 mAb 2 VL1;

(6) the VH and VL Domains of hPD-1 mAb 2;

(C) (1) the three CDR_{HS} of the VH Domain of PD-1 mAb 3;

(2) the three CDR_{LS} of the VL Domain of PD-1 mAb 3;

(3) the three CDR_{HS} of the VH Domain of PD-1 mAb 3 and the three CDR_{LS} of the VL Domain of PD-1 mAb 3;

(D) (1) the three CDR_{HS} of the VH Domain of PD-1 mAb 4;

(2) the three CDR_{LS} of the VL Domain of PD-1 mAb 4;

(3) the three CDR_{HS} of the VH Domain of PD-1 mAb 4 and the three CDR_{LS} of the VL Domain of PD-1 mAb 4;

(E) (1) the three CDR_{HS} of the VH Domain of PD-1 mAb 5;

(2) the three CDR_{LS} of the VL Domain of PD-1 mAb 5;

(3) the three CDR_{HS} of the VH Domain of PD-1 mAb 5 and the three CDR_{LS} of the VL Domain of PD-1 mAb 5;

(F) (1) the three CDR_{HS} of the VH Domain of PD-1 mAb 6;

(2) the three CDR_{LS} of the VL Domain of PD-1 mAb 6;

(3) the three CDR_{HS} of the VH Domain of PD-1 mAb 6 and the three CDR_{LS} of the VL Domain of PD-1 mAb 6;

(G) (1) the three CDR_{HS} of the VH Domain of PD-1 mAb 7;

(2) the three CDR_{LS} of the VL Domain of PD-1 mAb 7, or hPD-1 mAb 7 VL2, or hPD-1 mAb 7 VL3;

(3) the three CDR_{HS} of the VH Domain of PD-1 mAb 7 and the three CDR_{LS} of the VL Domain of PD-1 mAb 7, or hPD-1 mAb 7 VL2, hPD-1 mAb 7 VL3;

(4) the VH Domain of hPD-1 mAb 7 VH1, or hPD-1 mAb 7 VH2;

(5) the VL Domain of hPD-1 mAb 7 VL1, or hPD-1 mAb 7 VL2, or hPD-1 mAb 7 VL 3;

(6) the VH and VL Domains of the hPD-1 mAb 7(1.1), or hPD-1 mAb 7(1.2), or hPD-1 mAb 7(1.3), or hPD-1 mAb 7(2.1), or hPD-1 mAb 7(2.2), or hPD-1 mAb 7(2.3);

(H) (1) the three CDR_{HS} of the VH Domain of PD-1 mAb 8;

(2) the three CDR_{LS} of the VL Domain of PD-1 mAb 8;

(3) the three CDR_{HS} of the VH Domain of PD-1 mAb 8 and the three CDR_{LS} of the VL Domain of PD-1 mAb 8;

(I) (1) the three CDR_{HS} of the VH Domain of PD-1 mAb 9, or hPD-1 mAb 9 VH2;

(2) the three CDR_{LS} of the VL Domain of PD-1 mAb 9, or hPD-1 mAb 9 VL2;

(3) the three CDR_{HS} of the VH Domain of PD-1 mAb 9, or hPD-1 mAb 9 VH2 and the three CDR_{LS} of the VL Domain of PD-1 mAb 9, or hPD-1 mAb 9 VL2;

(4) the VH Domain of hPD-1 mAb 9 VH1, or hPD-1 mAb 9 VH2;

(5) the VL Domain of hPD-1 mAb 9 VL1, or hPD-1 mAb 9 VL2;

(6) the VH and VL Domains of the hPD-1 mAb 9(1.1), or hPD-1 mAb 9(1.2), or hPD-1 mAb 9(2.1), or hPD-1 mAb 9(2.2);

(J) (1) the three CDR_{HS} of the VH Domain of PD-1 mAb 10;

(2) the three CDR_{LS} of the VL Domain of PD-1 mAb 10;

(3) the three CDR_{HS} of the VH Domain of PD-1 mAb 10 and the three CDR_{LS} of the VL Domain of PD-1 mAb 10;

(K) (1) the three CDR_{HS} of the VH Domain of PD-1 mAb 11;

(2) the three CDR_{LS} of the VL Domain of PD-1 mAb 11;

(3) the three CDR_{HS} of the VH Domain of PD-1 mAb 11 and the three CDR_{LS} of the VL Domain of PD-1 mAb 11;

(L) (1) the three CDR_{HS} of the VH Domain of PD-1 mAb 12;

(2) the three CDR_{LS} of the VL Domain of the PD-1 mAb 12;

(3) the three CDR_{HS} of the VH Domain of the PD-1 mAb 12 and the three CDR_{LS} of the VL Domain of PD-1 mAb 12;

(M) (1) the three CDR_{HS} of the VH Domain of PD-1 mAb 13;

(2) the three CDR_{LS} of the VL Domain of PD-1 mAb 13;

(3) the three CDR_{HS} of the VH Domain of PD-1 mAb 13 and the three CDR_{LS} of the VL Domain of PD-1 mAb 13;

(N) (1) the three CDR_{HS} of the VH Domain of PD-1 mAb 14;

(2) the three CDR_{LS} of the VL Domain of the PD-1 mAb 14;

(3) the three CDR_{HS} of the VH Domain of the PD-1 mAb 14 and the three CDR_{LS} of the VL Domain of PD-1 mAb 14;

(O) (1) the three CDR_{HS} of the VH Domain of PD-1 mAb 15;

(2) the three CDR_{LS} of the VL Domain of PD-1 mAb 15;

(3) the three CDR_{HS} of the VH Domain of PD-1 mAb 15 and the three CDR_{LS} of the VL Domain of PD-1 mAb 15;

(4) the VH Domain of hPD-1 mAb 15 VH1;

(5) the VL Domain of hPD-1 mAb 15 VL1;

(6) the VH and VL Domains of hPD-1 mAb 15;

or

that binds, or competes for binding with, the same epitope as PD-1 mAb 1, PD-1 mAb 2, PD-1 mAb 3, PD-1 mAb 4, PD-1 mAb 5, PD-1 mAb 6, PD-1 mAb 7, PD-1 mAb 8, PD-1 mAb 9, PD-1 mAb 10, PD-1 mAb 11, PD-1 mAb 12, PD-1 mAb 13, PD-1 mAb 14, or PD-1 mAb 15.

A. The Anti-Human PD-1 Antibody PD-1 mAb 1

1. Murine Anti-Human PD-1 Antibody PD-1 mAb 1

[0089] The amino acid sequence of the VH Domain of PD-1 mAb 1 (**SEQ ID NO:69**) is shown below (CDR_H residues are shown underlined).

DVQLQESGPG RVKPSQSLSL TCTVTGFSIT **NDYAWN**WIRQ FPGNKLEWMG **HITYSGSTSY NPSLKS**RISI TRDTSKNHFF LQLSSVTPED TATYYCAR**DY GSGYPYTLDY** WGQGTSVTVS S

CDR_H1 of PD-1 mAb 1 (**SEQ ID NO:71**): **NDYAWN**

CDR_H2 of PD-1 mAb 1 (**SEQ ID NO:72**): **HITYSGSTSYNPSLKS**

CDR_H3 of PD-1 mAb 1 (**SEQ ID NO:73**): **DYGSGYPYTLDY**

[0090] An exemplary polynucleotide that encodes the VH Domain of PD-1 mAb 1 is **SEQ ID NO:70** (nucleotides encoding the CDR_H residues are shown underlined):

cagatccagt gatgtgcagc ttcaggagtc gggacctggc cgggtgaaac
cttctcagtc tctgtccctc acctgcactg tcactggctt ctcaatcacc
aatgattatg cctggaactg gatccgacag tttccaggaa acaaactgga
gtggatgggc **cacataacct acagtggcag cactagctac aacccatctc**
tcaaaagtcg aatctctatc actcgggaca catccaagaa ccacttcttc
ctgcagttga gttctgtgac tcctgaggac acagccacat attactgtgc

aagagattac ggtagtggct acccctatac tttggactac tggggtcaag
gtacctcagt caccgtctcc tcc

[0091] The amino acid sequence of the VL Domain of PD-1 mAb 1 (**SEQ ID NO:74**) is shown below (CDR_L residues are shown underlined):

QIVLTQSPAL MSASPGEKVT MTCSATSIVS YVYWYQQKPG SSPQPWIYLT
SNLASGVPAR FSGSGSGTSY SLTISSMEAE DAATYYCQQW SDNPYTFGGG
TKLEIK

CDR_L1 of PD-1 mAb 1 (**SEQ ID NO:76**): SATSIVSYVY

CDR_L2 of PD-1 mAb 1 (**SEQ ID NO:77**): LTSNLAS

CDR_L3 of PD-1 mAb 1 (**SEQ ID NO:78**): QQWSDNPYT

[0092] An exemplary polynucleotide that encodes the VL Domain of PD-1 mAb 1 is **SEQ ID NO:75** (nucleotides encoding the CDR_L residues are shown underlined):

caaattgttc tcacccagtc tccagcactc atgtctgcat ctccagggga
gaaggtcacc atgacctgca gtgccacctc aattgtaagt tacgtttact
ggtaccagca gaagcctgga tcctcccccc aaccctggat ttatctcaca
tccaacctgg cttctggagt ccctgctcgc ttcagtggca gtgggtctgg
gacctcttac tctctcacaa tcagcagcat ggaggctgaa gatgctgcca
cttattactg ccagcagtgg agtgataacc cgtacacgtt cggaggggg
accaagctgg aaataaaa

2. Humanization of the Anti-Human PD-1 Antibody PD-1 mAb 1 to Form "hPD-1 mAb 1"

[0093] The above-described murine anti-human PD-1 antibody PD-1 mAb 1 was humanized and further deimmunized when antigenic epitopes were identified in order to demonstrate the capability of humanizing an anti-human PD-1 antibody so as to decrease its antigenicity upon administration to a human recipient. The humanization yielded one humanized VH Domain, designated herein as "**hPD-1 mAb 1 VH1**," and one humanized VL Domain designated herein as "**hPD-1 mAb 1 VL1.**" Accordingly, an antibody comprising the humanized VL Domains paired with the humanized VH Domain is referred to as "**hPD-1 mAb 1**."

[0094] The amino acid sequence of the VH Domain of hPD-1 mAb 1 VH1 (**SEQ ID NO:79**) is shown below (CDR_H residues are shown underlined):

DVQLQESGPG LVKPSQTLSL TCTVSGFSIS NDYAWNWIRQ PPGKGLEWIG
HITYSGSTSY NPSLKSRLTI TRDTSKNQFV LTMTNMDPVD TATYYCARDY
GSGYPYTLDY WGQGTTVTVS S

[0095] An exemplary polynucleotide that encodes hPD-1 mAb 1 VH1 is **SEQ ID NO:80** (nucleotides encoding the CDR_H residues are shown underlined):

gacgtacagc tccaggaaag tggcccaggt ctggtgaagc catcccagac
actgagcctg acttgcaccg tgagtggctt ctccatctca aatgactacg
cctggaattg gattaggcag cctcccggta aagggctgga gtggatcggc
cacatcacat acagcggctc cacatcatat aatcccagtc tgaagagccg
tcttaccatt actcgcgaca ctagtaagaa ccagtttgtt ctgaccatga
ccaacatgga ccctgtggat actgcaacat actattgtgc tcgagattat
ggttctggtt acccttatac actcgactac tggggacagg gaaccactgt
gaccgtgagc tcc

[0096] The amino acid sequence of the VL Domain of hPD-1 mAb 1 VL1 (**SEQ ID NO:81**) is shown below (CDR_H residues are shown underlined):

EIVLTQSPAT LSVSPGEKVT ITCSATSIVS YVYWYQQKPG QAPQPLIYLT
SNLASGIPAR FSGSGSGTDF TLTISSLEAE DAATYYCQQW SDNPYTFGGG
TKVEIK

[0097] An exemplary polynucleotide that encodes hPD-1 mAb 1 VL1 is **SEQ ID NO:82** (nucleotides encoding the CDR_H residues are shown underlined):

gaaatcgttc tgacccagag cccagcaacc ctgtctgtct cccccggaga
aaaggtcacc attacttgct ctgctacttc tatcgtgtcc tacgtgtact
ggtatcagca gaagcccggt caggctcccc agccattgat atatctgacc
agcaacctgg cttctggtat cccagctcgt ttttccggta gcgggtccgg
gactgatttc actttgacta tcagctctct ggaggcagaa gacgccgcca
cctattattg tcaacagtgg tcagacaatc catacacttt tggcggtggc
accaaagtcg aaataaag

B. The Anti-Human PD-1 Antibody PD-1 mAb 2

1. Murine Anti-Human PD-1 Antibody PD-1 mAb 2

[0098] The amino acid sequence of the VH Domain of PD-1 mAb 2 (**SEQ ID NO:83**) is shown below (CDR_H residues are shown underlined).

DVQLVESGGG LVQPGGSRKL SCAASGFVFS SFGMHWVRQA PEKGLEWVAY
ISSGSMSISY ADTVKGRFTV TRDNAKNTLF LQMTSLRSED TAIYYCASLS
DYFDYWGQGT TLTVSS

CDR_H1 of PD-1 mAb 2 (**SEQ ID NO:85**): SFGMH

CDR_H2 of PD-1 mAb 2 (**SEQ ID NO:86**): YISSGSMSISYADTVKG

CDR_H3 of PD-1 mAb 2 (**SEQ ID NO:87**): LSDYFDY

[0099] An exemplary polynucleotide that encodes the VH Domain of PD-1 mAb 2 is **SEQ ID NO:84** (nucleotides encoding the CDR_H residues are shown underlined):

gatgtgcagc tcgtggagtc tgggggaggc ttagtgcagc ctggagggtc
ccggaaactc tcctgtgcag cctctggatt cgttttcagt **agctttggaa**
tgcactgggt tcgtcaggct ccagagaagg ggctggagtg ggtcgca**tac**
atcagtagtg gcagtatgag catttcctat gcagacacag tgaagggccg
attcaccgtc accagagaca atgccaagaa caccctgttc ctgcaaatga
ccagtctaag gtctgaggac acggccattt attactgtgc atcc**ctgagt**
gactactttg actactgggg ccaaggcacc actctcacag tctcctcc

[00100] The amino acid sequence of the VL Domain of PD-1 mAb 2 (**SEQ ID NO:88**) is shown below (CDR_L residues are shown underlined):

DVVMSQTPLS LPVSLGDQAS ISC**RSSQSLV HSTGNTYLH**W YLQKPGQSPK
LLIY**RVSNRF S**GVPDRFSGS GSGTDFTLKI SRVEAEDLGV FFC**SQTTHVP**
WTFGGGTKLE IK

CDR_L1 of PD-1 mAb 2 (**SEQ ID NO:90**): RSSQSLVHSTGNTYLH

CDR_L2 of PD-1 mAb 2 (**SEQ ID NO:91**): RVSNRFS

CDR_L3 of PD-1 mAb 2 (**SEQ ID NO:92**): SQTTHVPWT

[00101] An exemplary polynucleotide that encodes the VL Domain of PD-1 mAb 2 is **SEQ ID NO:89** (nucleotides encoding the CDR_L residues are shown underlined):

gatgttgtga tgtcccaaac tccactctcc ctgcctgtca gtcttggaga
tcaagcctcc atctcttgc**a gatctagtca gagccttgtt cacagtactg**
gaaacaccta tttacattgg tacctgcaga agccaggcca gtctccaaag
ctcctgatct ac**agggtttc taaccgattt tct**ggggtcc ccgacaggtt
cagtggcagt ggatcaggga cagatttcac actcaagatc agtagagtgg
aggctgagga tctgggagtt tttttctgc**t ctcaaactac acatgttccg**
tggacgttcg gtggaggcac caagctggaa atcaaa

2. Humanization of the Anti-Human PD-1 Antibody PD-1 mAb 2 to Form "hPD-1 mAb 2"

[00102] The above-described murine anti-human PD-1 antibody PD-1 mAb 2 was humanized and further deimmunized when antigenic epitopes were identified in order to demonstrate the capability of humanizing an anti-human PD-1 antibody so as to decrease its antigenicity upon administration to a human recipient. The humanization yielded one humanized VH Domain, designated herein as "**hPD-1 mAb 2 VH1,**" and one humanized VL Domains designated herein as "**hPD-1 mAb 1 VL1**." Accordingly, any antibody comprising

the humanized VL Domains paired with the humanized VH Domain is referred to as "**hPD-1 mAb 2**."

[00103] The amino acid sequence of the VH Domain of hPD-1 mAb 2 VH1 (**SEQ ID NO:93** is shown below (CDR_H residues are shown underlined):

EVQLVESGGG LVQPGGSLRL SCAASGFVFS **SFGMH**WVRQA PGKGLEWVA**Y**
ISSGSMSISY ADTVKGRFTI SRDNAKNTLY LQMNSLRTED TALYYCAS**LS**
DYFDYWGQGT TVTVSS

[00104] An exemplary polynucleotide that encodes hPD-1 mAb 2 VH1 is **SEQ ID NO:94** (nucleotides encoding the CDR_H residues are shown underlined):

gaagtgcaat tggttgagag tggtggtggc ctggtgcagc caggtggaag
tctgcggttg tcctgtgcag caagcggatt tgtgttcagc **tcttttggga**
tgcattgggt gcgccaggct cccggcaagg gtctcgagtg ggtagca**tac**
atctccagcg ggtccatgtc tattagttat gccgacacag tgaaaggcag
gtttactatc tcccgtgaca atgcaaaaaa cacactgtac ctgcaaatga
atagcctgcg caccgaggac accgccttgt actactgcgc ttcc**ctgtct**
gattacttcg actactgggg tcagggcaca actgtgacag tttcttcc

[00105] The amino acid sequence of the VL Domain of hPD-1 mAb 2 VL1 (**SEQ ID NO:95** is shown below (CDR_H residues are shown underlined):

DVVMTQSPLS LPVTLGQPAS ISC**RSSQSLV HSTGNTYLH**W YLQKPGQSPQ
LLIY**RVSNRF S**GVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYC**SQTTHVP**
WTFGQGTKLE IK

[00106] An exemplary polynucleotide that encodes hPD-1 mAb 2 VL1 is **SEQ ID NO:96** (nucleotides encoding the CDR_H residues are shown underlined):

gacgttgtga tgacacagtc accactgagt ctgccagtta ccctgggcca
gccagccagt atttcttgt**c ggagttcaca gagtctggta cattccacag**
gaaatacata tctccattgg tacctgcaaa aaccagggca gagcccccag
ctgctgattt at**agagtgtc taatcgattt tct**ggcgtgc cagatcggtt
cagcggcagc gggtctggca ctgatttcac actgaaaatc tctagggtgg
aggcagagga cgtaggcgtt tactactgt**a gtcagaccac ccatgtaccc**
tggacttttg gccaaggtac taagctggaa atcaag

C. Murine Anti-Human PD-1 Antibody PD-1 mAb 3

[00107] The amino acid sequence of the VH Domain of PD-1 mAb 3 (**SEQ ID NO:97**) is shown below (CDR_H residues are shown underlined).

QVQLQQSGAE LVRPGASVTL SCKASGYTFT **DYVMH**WVKQT PVHGLEWIG**T**
IDPETGGTAY NQKFKGKAIL TADKSSNTAY MELRSLTSED SAVYYFTR**EK**
ITTIVEGTYW YFDVWGTGTT VTVSS

CDR_H1 of PD-1 mAb 3 (**SEQ ID NO:99**): DYVMH

CDR_H2 of PD-1 mAb 3 (**SEQ ID NO:100**): TIDPETGGTAYNQKFKG

CDR_H3 of PD-1 mAb 3 (**SEQ ID NO:101**): EKITTIVEGTYWYFDV

[00108] An exemplary polynucleotide that encodes the VH Domain of PD-1 mAb 3 is **SEQ ID NO:98** (nucleotides encoding the CDR_H residues are shown underlined):

caggttcaac tgcaacagtc tggggctgag ctggtgaggc ctggggcttc
agtgacgctg tcctgcaagg cttcgggcta cacatttact gactatgtaa
tgcactgggt gaagcagaca cctgtgcatg gcctggaatg gattggaact
attgatcctg aaactggtgg tactgcctac aatcagaagt tcaagggcaa
ggccatactg actgcagaca agtcctccaa cacagcctac atggagctcc
gcagcctgac atctgaggac tctgccgtct attactttac aagagagaag
attactacga tagtagaggg gacatactgg tacttcgatg tctggggcac
agggaccacg gtcaccgtct cctca

[00109] The amino acid sequence of the VL Domain of PD-1 mAb 3 (**SEQ ID NO:102**) is shown below (CDR_L residues are shown underlined):

DVLLTQTPLS LPVSLGDQAS ISCRSSQNIV HSNGDTYLEW YLQKPGQSPK
LLIYKVSNRF SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YYCFQGSHLP
YTFGGGTKLE IK

CDR_L1 of PD-1 mAb 3 (**SEQ ID NO:104**): RSSQNIVHSNGDTYLE

CDR_L2 of PD-1 mAb 3 (**SEQ ID NO:105**): KVSNRFS

CDR_L3 of PD-1 mAb 3 (**SEQ ID NO:106**): FQGSHLPYT

[00110] An exemplary polynucleotide that encodes the VL Domain of PD-1 mAb 3 is **SEQ ID NO:103** (nucleotides encoding the CDR_L residues are shown underlined):

gatgttttgc tgacccaaac tccactctcc ctgcctgtca gtcttggaga
tcaagcctcc atctcttgca gatctagtca gaacattgta catagtaatg
gagacaccta tttggaatgg tacctgcaga aaccaggcca gtctccaaag
ctcctgatct ataaagtttc caaccgattt tctggggtcc cagacaggtt
cagtggcagt gggtcaggga cagattttac actcaaaatc agcagagtgg
aggctgagga tctgggagtt tattactgct ttcaaggttc acatcttccg
tacacgttcg gaggggggac caagctggaa ataaaa

D. Murine Anti-Human PD-1 Antibody PD-1 mAb 4

[00111] The amino acid sequence of the VH Domain of PD-1 mAb 4 (**SEQ ID NO:107**) is shown below (CDR_H residues are shown underlined).

DVQLVESGGG LVQPGGSRKL SCAASGFVFS **SFGMH**WVRQA PEKGLEWVA**Y**
ISSGSMSISY ADTVKGRFTV TRDNAKNTLF LQMTSLRSED TAIYYCAS**LT**
DYFDYWGQGT TLTVSS

CDR$_H$1 of PD-1 mAb 4 (**SEQ ID NO:109**): **SFGMH**

CDR$_H$2 of PD-1 mAb 4 (**SEQ ID NO:110**): **YISSGSMSISYADTVKG**

CDR$_H$3 of PD-1 mAb 4 (**SEQ ID NO:111**): **LTDYFDY**

[00112] An exemplary polynucleotide that encodes the VH Domain of PD-1 mAb 4 is **SEQ ID NO:108** (nucleotides encoding the CDR$_H$ residues are shown underlined):

gatgtgcagc tcgtggagtc tgggggaggc ttagtgcagc ctggagggtc
ccggaaactc tcctgtgcag cctctggatt cgttttcagt **agctttggaa**
tgcactgggt tcgtcaggct ccagagaagg ggctggagtg ggtcgca**tat**
attagtagtg gcagtatgag tatttcctat gcagacacag tgaagggccg
attcaccgtc accagagaca atgccaagaa caccctgttc ctgcaaatga
ccagtctaag gtctgaggac acggccattt attactgtgc atcc**ctgact**
gactactttg actactgggg ccaaggcacc actctcacag tctcctca

[00113] The amino acid sequence of the VL Domain of PD-1 mAb 4 (**SEQ ID NO:112**) is shown below (CDR$_L$ residues are shown underlined):

DVVMSQTPLS LPVSLGDQAS ISC**RSSQSLV HSTGNTYFH**W YLQKPGQSPK
LLIY**RVSNRF S**GVPDRFSGS GSGTDFTLKI SRVEAEDLGV YFC**SQTTHVP**
WTFGGGTKLE IK

CDR$_L$1 of PD-1 mAb 4 (**SEQ ID NO:114**): **RSSQSLVHSTGNTYFH**

CDR$_L$2 of PD-1 mAb 4 (**SEQ ID NO:115**): **RVSNRFS**

CDR$_L$3 of PD-1 mAb 4 (**SEQ ID NO:116**): **SQTTHVPWT**

[00114] An exemplary polynucleotide that encodes the VL Domain of PD-1 mAb 4 is **SEQ ID NO:113** (nucleotides encoding the CDR$_L$ residues are shown underlined):

gatgttgtga tgtcccaaac tccactctcc ctgcctgtca gtcttggaga
tcaagcctcc atctcctgc**a gatctagtca gagccttgtt cacagtactg**
gaaacaccta tttccattgg tacctgcaga agccaggcca gtctccaaag
ctcctgatct ac**agggtttc taaccgattt tct**ggggtcc ccgacaggtt
cagtggcagt ggatcaggga cagatttcac actcaagatc agcagagtgg
aggctgagga tctgggagtt tatttctgc**t ctcaaactac acatgttccg**
tggacgttcg gtggaggcac caagctggaa atcaaa

E. Murine Anti-Human PD-1 Antibody PD-1 mAb 5

[00115] The amino acid sequence of the VH Domain of PD-1 mAb 5 (**SEQ ID NO:117**) is shown below (CDR_H residues are shown underlined).

QVQLQQPGVE LVRPGASVKL SCKASGYSFT **AYWMN**WMKQR PGQGLEWIG**V**
IHPSDSETWL NQKFKDKATL TVDKSSSTAY MQLISPTSED SAVYYCAR**EH**
YGSSPFAYWG QGTLVTVSA

CDR_H1 of PD-1 mAb 5 (**SEQ ID NO:119**): **AYWMN**

CDR_H2 of PD-1 mAb 5 (**SEQ ID NO:120**): **VIHPSDSETWLNQKFKD**

CDR_H3 of PD-1 mAb 5 (**SEQ ID NO:121**): **EHYGSSPFAY**

[00116] An exemplary polynucleotide that encodes the VH Domain of PD-1 mAb 5 is **SEQ ID NO:118** (nucleotides encoding the CDR_H residues are shown underlined):

caggtccaac tgcagcagcc tggggttgaa ctggtgaggc ctggagcttc
agtgaagctg tcctgcaagg cttctggcta ctccttcacc **gcctactgga**
tgaactggat gaaacagagg cctggacaag gccttgagtg gattggc**gtg**
attcatcctt ccgatagtga aacttggtta aatcagaagt tcaaggacaa
ggccacattg actgtagaca aatcctccag cacagcctac atgcaactca
tcagcccgac atctgaggac tctgcggtct attactgtgc aaga**gagcac**
tacggtagta gcccgtttgc ttactggggc caagggactc tggtcactgt
ctctgca

[00117] The amino acid sequence of the VL Domain of PD-1 mAb 5 (**SEQ ID NO:122**) is shown below (CDR_L residues are shown underlined):

DIVLTQSPAS LAVSLGQRAT ISC**RANESVD NYGMSFMN**WF QQKPGQPPKL
LIY**AASNQGS** GVPARFSGSG SGTDFSLNIH PMEEDDTAMY FC**QQSKEVPY**
TFGGGTKLEI K

CDR_L1 of PD-1 mAb 5 (**SEQ ID NO:124**): **RANESVDNYGMSFMN**

CDR_L2 of PD-1 mAb 5 (**SEQ ID NO:125**): **AASNQGS**

CDR_L3 of PD-1 mAb 5 (**SEQ ID NO:126**): **QQSKEVPYT**

[00118] An exemplary polynucleotide that encodes the VL Domain of PD-1 mAb 5 is **SEQ ID NO:123** (nucleotides encoding the CDR_L residues are shown underlined):

gacattgtgc tgacccaatc tccagcttct ttggctgtgt ctctagggca
gagggccacc atctcctgc**a gagccaacga aagtgttgat aattatggca**
tgagttttat gaactggttc caacagaaac caggacagcc acccaaactc
ctcatctat**g ctgcatccaa ccaaggatcc** ggggtccctg ccaggtttag
tggcagtggg tctgggacag atttcagcct caacatccat cctatggagg

aggatgatac tgcaatgtat ttctgtcagc aaagtaagga ggttccgtac acgttcggag gggggaccaa gctggaaata aaa

F. Murine Anti-Human PD-1 Antibody PD-1 mAb 6

[00119] The amino acid sequence of the VH Domain of PD-1 mAb 6 (**SEQ ID NO:127**) is shown below (CDR_H residues are shown underlined).

EVKLVESGGG LVNPGGSLKL SCAASGFTFS SYGMSWVRQT PEKRLEWVAT ISGGGSDTYY PDSVKGRFTI SRDNAKNNLY LQMSSLRSED TALYYCARQK ATTWFAYWGQ GTLVTVST

CDR_H1 of PD-1 mAb 6 (**SEQ ID NO:129**): SYGMS

CDR_H2 of PD-1 mAb 6 (**SEQ ID NO:130**): TISGGGSDTYYPDSVKG

CDR_H3 of PD-1 mAb 6 (**SEQ ID NO:131**): QKATTWFAY

[00120] An exemplary polynucleotide that encodes the VH Domain of PD-1 mAb 6 is **SEQ ID NO:128** (nucleotides encoding the CDR_H residues are shown underlined):

gaaatcgtac tcacccagtc acctgcaacc ctttctctga gccccggtga acgtgccact ctcagctgca gagcaagtga gagtgtggac aattacggca tgtccttcat gaactggttt cagcagaagc ctgggcagcc acctaagctg ctcatccacg ccgcctctaa ccgcggatct ggggtgcctt cacgtttttc tggatcagga agtggcactg acttcaccct tacaatcagc tctctggagc cagaggactt tgccgtctat ttctgccagc aatctaaaga ggtgccctat acttttggtg gcgggaccaa ggttgagatc aaa

[00121] The amino acid sequence of the VL Domain of PD-1 mAb 6 (**SEQ ID NO:132**) is shown below (CDR_L residues are shown underlined):

DIVLTQSPAS LAVSLGQRAT ISCRASESVD NYGISFMNWF QQKPGQPPKL LIYPASNQGS GVPARFSGSG SGTDFSLNIH PMEEDDAAMY FCQQSKEVPW TFGGGTKLEI K

CDR_L1 of PD-1 mAb 6 (**SEQ ID NO:134**): RASESVDNYGISFMN

CDR_L2 of PD-1 mAb 6 (**SEQ ID NO:135**): PASNQGS

CDR_L3 of PD-1 mAb 6 (**SEQ ID NO:136**): QQSKEVPWT

[00122] An exemplary polynucleotide that encodes the VL Domain of PD-1 mAb 6 is **SEQ ID NO:133** (nucleotides encoding the CDR_L residues are shown underlined):

gacattgtgc tgacccaatc tccagcttct ttggctgtgt ctctagggca gagggccacc atctcctgca gagccagcga aagtgttgat aattatggca ttagttttat gaactggttc caacagaaac caggacagcc acccaaactc ctcatctatc ctgcatccaa ccaaggatcc ggggtccctg ccaggtttag

tggcagtggg tctgggacag acttcagcct caacatccat cctatggagg aggatgatgc tgcaatgtat ttctgtcagc aaagtaagga ggttccgtgg acgttcggtg gaggcaccaa gctggaaatc aaa

G. The Anti-Human PD-1 Antibody PD-1 mAb 7

1. Murine Anti-Human PD-1 Antibody PD-1 mAb 7

[00123] The amino acid sequence of the VH Domain of PD-1 mAb 7 (**SEQ ID NO:137**) is shown below (CDR_H residues are shown underlined).

QVQLQQPGAE LVRPGASVKL SCKASGYSFT SYWMNWVKQR PGQGLEWIGV IHPSDSETWL DQKFKDKATL TVDKSSTTAY MQLISPTSED SAVYYCAREH YGTSPFAYWG QGTLVTVSS

CDR_H1 of PD-1 mAb 7 (**SEQ ID NO:139**): SYWMN

CDR_H2 of PD-1 mAb 7 (**SEQ ID NO:140**): VIHPSDSETWLDQKFKD

CDR_H3 of PD-1 mAb 7 (**SEQ ID NO:141**): EHYGTSPFAY

[00124] An exemplary polynucleotide that encodes the VH Domain of PD-1 mAb 7 is **SEQ ID NO:138** (nucleotides encoding the CDR_H residues are shown underlined):

gaggtccaac tgcagcagcc tggggctgaa ctggtgaggc ctggagcttc agtgaagctg tcctgcaagg cttctggcta ctccttcacc agctactgga tgaactgggt gaagcagagg cctggacaag gccttgagtg gattggcgtg attcatcctt ccgatagtga aacttggtta gatcagaagt tcaaggacaa ggccacattg actgtagaca aatcctccac cacagcctac atgcaactca tcagcccgac atctgaggac tctgcggtct attactgtgc aagggagcac tacggtacta gcccgtttgc ttactggggc caagggactc tggtcactgt gtcttcc

[00125] The amino acid sequence of the VL Domain of PD-1 mAb 7 (**SEQ ID NO:142**) is shown below (CDR_L residues are shown underlined):

DIVLTQSPAS LAVSLGQRAT ISCRANESVD NYGMSFMNWF QQKPGQPPKL LIHAASNQGS GVPARFSGSG FGTDFSLNIH PMEEDDAAMY FCQQSKEVPY TFGGGTKLEI K

CDR_L1 of PD-1 mAb 7 (**SEQ ID NO:144**): RANESVDNYGMSFMN

CDR_L2 of PD-1 mAb 7 (**SEQ ID NO:145**): AASNQGS

CDR_L3 of PD-1 mAb 7 (**SEQ ID NO:146**): QQSKEVPYT

[00126] An exemplary polynucleotide that encodes the VL Domain of PD-1 mAb 7 is **SEQ ID NO:143** (nucleotides encoding the CDR_L residues are shown underlined):

gacattgtgc tgacccaatc tccagcttct ttggctgtgt ctctagggca
gagggccacc atctcctgca gagccaacga aagtgttgat aattatggca
tgagttttat gaactggttc caacagaaac caggacagcc acccaaactc
ctcatccatg ctgcatccaa ccaaggatcc ggggtccctg ccaggtttag
tggcagtggg tttgggacag acttcagcct caacatccat cctatggagg
aggatgatgc tgcaatgtat ttctgtcagc aaagtaagga ggttccgtac
acgttcggag gggggaccaa gctggaaata aaa

2. Humanization of the Anti-Human PD-1 Antibody PD-1 mAb 7 to Form "hPD-1 mAb 7"

[00127] The above-described murine anti-human PD-1 antibody PD-1 mAb 7 was humanized and further deimmunized when antigenic epitopes were identified in order to demonstrate the capability of humanizing an anti-human PD-1 antibody so as to decrease its antigenicity upon administration to a human recipient. The humanization yielded two humanized VH Domains, designated herein as "**hPD-1 mAb 7 VH1,**" and "**hPD-1 mAb 7 VH2**," and three humanized VL Domains designated herein as "**hPD-1 mAb 7 VL1**," "**hPD-1 mAb 7 VL2**," and "**hPD-1 mAb 7 VL3**." Any of the humanized VL Domains may be paired with either of the humanized VH Domains. Accordingly, any antibody comprising one of the humanized VL Domains paired with the humanized VH Domain is referred to generically as "**hPD-1 mAb 7**," and particular combinations of humanized VH/VL Domains are referred to by reference to the specific VH/VL Domains, for example a humanized antibody comprising hPD-1 mAb 7 VH1 and hPD-1 mAb 1 VL2 is specifically referred to as "**hPD-1 mAb 7(1.2)**."

[00128] The amino acid sequence of the VH Domain of hPD-1 mAb 7 VH1 (**SEQ ID NO:147**) is shown below (CDR_H residues are shown underlined):

QVQLVQSGAE VKKPGASVKV SCKASGYSFT SYWMNWVRQA PGQGLEWIGV
IHPSDSETWL DQKFKDRVTI TVDKSTSTAY MELSSLRSED TAVYYCAREH
YGTSPFAYWG QGTLVTVSS

[00129] An exemplary polynucleotide that encodes hPD-1 mAb 7 VH1 is **SEQ ID NO:148** (nucleotides encoding the CDR_H residues are shown underlined):

caagttcaat tggtacagag cggggcagag gtgaagaaac ccggcgccag
tgttaaggtg tcctgcaaag ccagcggtta cagctttaca agctattgga
tgaattgggt gcgtcaagca ccagggcagg gtctggaatg gattgggtg
atacatcctt ctgacagcga aacatggttg gaccagaaat ttaaagatcg
tgtgacaatt acagtcgata agtccacaag cactgcttac atggaactct
ccagcttgcg gtccgaggac accgctgtgt attattgcgc cagagagcac

tacggcacat caccttttgc atactggggc cagggaactc tcgtaaccgt atcctcc

[00130] The amino acid sequence of the VH Domain of hPD-1 mAb 7 VH2 (**SEQ ID NO:149**) is shown below (CDR_H residues are shown underlined):

QVQLVQSGAE VKKPGASVKV SCKASGYSFT SYWMNWVRQA PGQGLEWAGV IHPSDSETWL DQKFKDRVTI TVDKSTSTAY MELSSLRSED TAVYYCAREH YGTSPFAYWG QGTLVTVSS

[00131] An exemplary polynucleotide that encodes hPD-1 mAb 7 VH2 is **SEQ ID NO:150** (nucleotides encoding the CDR_H residues are shown underlined):

caagttcaat tggtacagag cggggcagag gtgaagaaac ccggcgccag tgttaaggtg tcctgcaaag ccagcggtta cagctttaca agctattgga tgaattgggt gcgtcaagca ccagggcagg gtctggaatg ggctggggtg atacatcctt ctgacagcga aacatggttg gaccagaaat ttaaagatcg tgtgacaatt acagtcgata agtccacaag cactgcttac atggaactct ccagcttgcg gtccgaggac accgctgtgt attattgcgc cagagagcac tacggcacat caccttttgc atactggggc cagggaactc tcgtaaccgt atcctcc

[00132] The amino acid sequence of the VL Domain of hPD-1 mAb 7 VL1 (**SEQ ID NO:151**) is shown below (CDR_H residues are shown underlined):

EIVLTQSPAT LSLSPGERAT LSCRANESVD NYGMSFMNWF QQKPGQPPKL LIHAASNQGS GVPSRFSGSG SGTDFTLTIS SLEPEDFAVY FCQQSKEVPY TFGGGTKVEI K

[00133] An exemplary polynucleotide that encodes hPD-1 mAb 7 VL1 is **SEQ ID NO:152** (nucleotides encoding the CDR_H residues are shown underlined):

gaaatcgtac tcacccagtc acctgcaacc ctttctctga gccccggtga acgtgccact ctcagctgca gagcaaatga gagtgtggac aattacggca tgtccttcat gaactggttt cagcagaagc ctgggcagcc acctaagctg ctcatccacg ccgcctctaa ccagggatct ggggtgcctt cacgtttttc tggatcagga agtggcactg acttcaccct tacaatcagc tctctggagc cagaggactt tgccgtctat ttctgccagc aatctaaaga ggtgccctat actttggtg gcgggaccaa ggttgagatc aaa

[00134] The amino acid sequence of the VL Domain of hPD-1 mAb 7 VL2 (**SEQ ID NO:153**) is shown below (CDR_H residues are shown underlined):

EIVLTQSPAT LSLSPGERAT LSCRASESVD NYGMSFMNWF QQKPGQPPKL LIHAASNQGS GVPSRFSGSG SGTDFTLTIS SLEPEDFAVY FCQQSKEVPY TFGGGTKVEI K

[00135] An exemplary polynucleotide that encodes hPD-1 mAb 7 VL2 is **SEQ ID NO:154** (nucleotides encoding the CDR_H residues are shown underlined):

gaaatcgtac tcacccagtc acctgcaacc ctttctctga gccccggtga
acgtgccact ctcagctgca gagcaagtga gagtgtggac aattacggca
tgtccttcat gaactggttt cagcagaagc ctgggcagcc acctaagctg
ctcatccacg ccgcctctaa ccagggatct ggggtgcctt cacgtttttc
tggatcagga agtggcactg acttcaccct tacaatcagc tctctggagc
cagaggactt tgccgtctat ttctgccagc aatctaaaga ggtgccctat
acttttggtg gcgggaccaa ggttgagatc aaa

[00136] The amino acid sequence of the VL Domain of hPD-1 mAb 7 VL3 (**SEQ ID NO:155**) is shown below (CDR_H residues are shown underlined):

EIVLTQSPAT LSLSPGERAT LSCRASESVD NYGMSFMNWF QQKPGQPPKL
LIHAASNRGS GVPSRFSGSG SGTDFTLTIS SLEPEDFAVY FCQQSKEVPY
TFGGGTKVEI K

[00137] An exemplary polynucleotide that encodes hPD-1 mAb 7 VL3 is **SEQ ID NO:156** (nucleotides encoding the CDR_H residues are shown underlined):

gaaatcgtac tcacccagtc acctgcaacc ctttctctga gccccggtga
acgtgccact ctcagctgca gagcaagtga gagtgtggac aattacggca
tgtccttcat gaactggttt cagcagaagc ctgggcagcc acctaagctg
ctcatccacg ccgcctctaa ccgcggatct ggggtgcctt cacgtttttc
tggatcagga agtggcactg acttcaccct tacaatcagc tctctggagc
cagaggactt tgccgtctat ttctgccagc aatctaaaga ggtgccctat
acttttggtg gcgggaccaa ggttgagatc aaa

[00138] The CDR_L1 of the VL Domain of both hPD-1 mAb 7 VL2 and hPD-1 mAb 7 VL3 comprises an asparagine to serine amino acid substitution and has the amino acid sequence: RASESVDNYGMSFMN ((**SEQ ID NO:157**), the substituted serine is shown underlined). It is contemplated that a similar substitution may be incorporated into any of the PD-1 mAb 7 CDR_L1 Domains described above.

[00139] In addition, the CDR_L2 of the VL Domain of hPD-1 mAb 7 VL3 comprises a glutamine to arginine amino acid substitution and has the amino acid sequence: AASNRGS ((**SEQ ID NO:158**), the substituted arginine is shown underlined). It is contemplated that a similar substitution may be incorporated into any of the PD-1 mAb 7 CDR_L2 Domains described above.

H. Murine Anti-Human PD-1 Antibody PD-1 mAb 8

[00140] The amino acid sequence of the VH Domain of PD-1 mAb 8 (**SEQ ID NO:159**) is shown below (CDR_H residues are shown underlined).

EGQLQQSGPE LVKPGASVKI SCKASGYTFT **DYYMN**WVKQN HGKSLEWIG**D**
INPKNGDTHY NQKFKGEATL TVDKSSTTAY MELRSLTSED SAVYYCAS**DF**
DYWGQGTTLT VSS

CDR_H1 of PD-1 mAb 8 (**SEQ ID NO:161**): **DYYMN**

CDR_H2 of PD-1 mAb 8 (**SEQ ID NO:162**): **DINPKNGDTHYNQKFKG**

CDR_H3 of PD-1 mAb 8 (**SEQ ID NO:163**): **DFDY**

[00141] An exemplary polynucleotide that encodes the VH Domain of PD-1 mAb 8 is **SEQ ID NO:160** (nucleotides encoding the CDR_H residues are shown underlined):

gagggccagc tgcaacaatc tggacctgag ctggtgaagc ctggggcttc
agtgaagata tcctgtaagg cttctggata cacgttcact **gactactaca**
tgaactgggt gaagcagaac catggaaaga gccttgagtg gattgga**gat**
attaatccta aaaatggtga cactcactac aaccagaagt tcaagggcga
ggccacattg actgtagaca agtcctccac cacagcctac atggagctcc
gcagcctgac atctgaggac tctgcagtct attactgtgc gagc**gatttt**
gactactggg gccaaggcac cactctcaca gtctcctcc

[00142] The amino acid sequence of the VL Domain of PD-1 mAb 8 (**SEQ ID NO:164**) is shown below (CDR_L residues are shown underlined):

DVVMTQTPLS LPVGLGDQAS ISC**RSSQTLV YSNGNTYLN**W FLQKPGQSPK
LLIY**KVSNRF S**GVPDRFSGS GSGTDFTLKI SRVEAEDLGV YFC**SQSTHVP**
FTFGSGTKLE IK

CDR_L1 of PD-1 mAb 8 (**SEQ ID NO:166**): **RSSQTLVYSNGNTYLN**

CDR_L2 of PD-1 mAb 8 (**SEQ ID NO:167**): **KVSNRFS**

CDR_L3 of PD-1 mAb 8 (**SEQ ID NO:168**): **SQSTHVPFT**

[00143] An exemplary polynucleotide that encodes the VL Domain of PD-1 mAb 8 is **SEQ ID NO:165** (nucleotides encoding the CDR_L residues are shown underlined):

gatgttgtga tgacccaaac tccactctcc ctgcctgtcg gtcttggaga
tcaagcctcc atctcttgc**a gatctagtca gacccttgta tatagtaatg**
gaaacaccta tttaaattgg ttcctgcaga agccaggcca gtctccaaag
ctcctgatct ac**aaagtttc caaccgattt tct**ggggtcc cagacaggtt
cagtggcagt ggatcaggga cagatttcac actcaagatc agcagagtgg
aggctgagga tctgggagtt tatttctgc**t ctcaaagtac acatgttcca**
ttcacgttcg gctcggggac aaagttggaa ataaaa

I. The Anti-Human PD-1 Antibody PD-1 mAb 9

1. Murine Anti-Human PD-1 Antibody PD-1 mAb 9

[00144] The amino acid sequence of the VH Domain of PD-1 mAb 9 (**SEQ ID NO:169**) is shown below (CDR_H residues are shown underlined).

EVMLVESGGG LVKPGGSLKL SCAASGFTFS **SYLVS**WVRQT PEKRLEWVA**T**
ISGGGGNTYY SDSVKGRFTI SRDNAKNTLY LQISSLRSED TALYYCAR**YG**
FDGAWFAYWG QGTLVTVSS

CDR_H1 of PD-1 mAb 9 (**SEQ ID NO:171**): **SYLVS**

CDR_H2 of PD-1 mAb 9 (**SEQ ID NO:172**): **TISGGGGNTYYSDSVKG**

CDR_H3 of PD-1 mAb 9 (**SEQ ID NO:173**): **YGFDGAWFAY**

[00145] An exemplary polynucleotide that encodes the VH Domain of PD-1 mAb 9 is **SEQ ID NO:170** (nucleotides encoding the CDR_H residues are shown underlined):

gaagtgatgc tggtggagtc tgggggaggc ttagtgaagc ctggagggtc
cctgaaactc tcctgtgcag cctctggatt cactttcagt **agttatcttg**
tgtcttgggt tcgccagact ccggagaaga ggctggagtg ggtcgcaacc
attagtggtg gtggtggtaa cacctactat tcagacagtg tgaagggtcg
attcaccatc tccagagaca atgccaagaa caccctgtac ctgcaaatca
gcagtctgag gtctgaggac acggccttgt attactgtgc aagg**tatggt**
ttcgacggcg cctggtttgc ttactggggc caagggactc tggtcactgt
ctcttcc

[00146] The amino acid sequence of the VL Domain of PD-1 mAb 9 (**SEQ ID NO:174**) is shown below (CDR_L residues are shown underlined):

DIQMTQSPAS LSASVGDIVT ITC**RASENIY SYLA**WYQQKQ EKSPQLLVY**N**
AKTLAAGVPS RFSGSGSGTQ FSLTINSLQP EDFGNYYC**QH HYAVPWT**FGG
GTRLEIT

CDR_L1 of PD-1 mAb 9 (**SEQ ID NO:176**): **RASENIYSYLA**

CDR_L2 of PD-1 mAb 9 (**SEQ ID NO:177**): **NAKTLAA**

CDR_L3 of PD-1 mAb 9 (**SEQ ID NO:178**): **QHHYAVPWT**

[00147] An exemplary polynucleotide that encodes the VL Domain of PD-1 mAb 9 is **SEQ ID NO:175** (nucleotides encoding the CDR_L residues are shown underlined):

gacatccaga tgactcagtc tccagcctcc ctatctgcat ctgtgggaga
tattgtcacc atcacatgt**c gagcaagtga gaatatttac agttatttag**
catggtatca gcagaaacag gaaaaatctc ctcagctcct ggtctat**aat**
gcaaaaacct tggcagcagg tgtgccatca aggttcagtg gcagtggatc

aggcacacag ttttctctga ccatcaacag cctgcagcct gaagattttg
ggaattatta ctgtcagcat cattatgctg ttccgtggac gttcggtgga
ggcaccagac tggaaatcac a

2. Humanization of the Anti-Human PD-1 Antibody PD-1 mAb 9 to Form "hPD-1 mAb 9"

[00148] The above-described murine anti-human PD-1 antibody PD-1 mAb 9 was humanized and further deimmunized when antigenic epitopes were identified in order to demonstrate the capability of humanizing an anti-human PD-1 antibody so as to decrease its antigenicity upon administration to a human recipient. The humanization yielded two humanized VH Domains, designated herein as "**hPD-1 mAb 9 VH1,**" and "**hPD-1 mAb 9 VH2**," and two humanized VL Domains designated herein as "**hPD-1 mAb 9 VL1**," and "**hPD-1 mAb 9 VL2**." Any of the humanized VL Domains may be paired with the humanized VH Domains. Accordingly, any antibody comprising one of the humanized VL Domains paired with the humanized VH Domain is referred to generically as "**hPD-1 mAb 9**," and particular combinations of humanized VH/VL Domains are referred to by reference to the specific VH/VL Domains, for example a humanized antibody comprising hPD-1 mAb 9 VH1 and hPD-1 mAb 9 VL2 is specifically referred to as "**hPD-1 mAb 9(1.2)**."

[00149] The amino acid sequence of the VH Domain of hPD-1 mAb 9 VH1 (**SEQ ID NO:179**) is shown below (CDR_H residues are shown underlined):

EVQLVESGGG LVRPGGSLKL SCAASGFTFS SYLVSWVRQA PGKGLEWVAT
ISGGGGNTYY SDSVKGRFTI SRDNAKNSLY LQMNSLRAED TATYYCARYG
FDGAWFAYWG QGTLVTVSS

[00150] An exemplary polynucleotide that encodes hPD-1 mAb 9 VH1 is **SEQ ID NO:180** (nucleotides encoding the CDR_H residues are shown underlined):

gaggtgcagc tggtggaaag tgggggcggc ctggtgcgac ccgggggaag
tctgaaactg tcctgtgcag catcaggatt tacttttca tcttatctcg
tgtcttgggt aagacaagca cccggaaaag gcttggaatg ggtggccact
atctccggtg gaggtggcaa cacctactat agcgacagtg tcaagggaag
atttaccatc agtcgcgaca acgctaagaa tagcctgtac ctccagatga
actccctgcg cgccgaggac accgccacct attactgtgc acgctatgga
tttgacggcg catggtttgc ctactgggga cagggcacat tggtaaccgt
tagctcc

[00151] The amino acid sequence of the VH Domain of hPD-1 mAb 9 VH2 (**SEQ ID NO:181**) is shown below (CDR_H residues are shown underlined):

EVQLVESGGG LARPGGSLKL SCAASGFTFS **SYLVG**WVRQA PGKGLEWTA**T**
ISGGGGNTYY SDSVKGRFTI SRDNAKNSLY LQMNSARAED TATYYCAR**YG**
FDGAWFAYWG QGTLVTVSS

[00152] An exemplary polynucleotide that encodes hPD-1 mAb 9 VH2 is **SEQ ID NO:182** (nucleotides encoding the CDR_H residues are shown underlined):

gaggtgcagc tggtggaaag tgggggcggc ctggcgcgac ccgggggaag
tctgaaactg tcctgtgcag catcaggatt tacttttca **tcttatctcg**
tgggctgggt aagacaagca cccggaaaag gcttggaatg gacggcc**act**
atctccggtg gaggtggcaa cacctactat agcgacagtg tcaagggaag
atttaccatc agtcgcgaca acgctaagaa tagcctgtac ctccagatga
actccgcacg cgccgaggac accgccacct attactgtgc acgc**tatgga**
tttgacggcg catggtttgc ctactgggga cagggcacat tggtaaccgt
tagctcc

[00153] The CDR_H1 of the VH Domain of hPD-1 mAb 9 VH2 comprises a serine to glycine amino acid substitution and has the amino acid sequence: SYLV**G** ((**SEQ ID NO:183**), the substituted glycine is shown underlined). It is contemplated that a similar substitution may be incorporated into any of the PD-1 mAb 9 CDR_H1 Domains described above.

[00154] The amino acid sequence of the VL Domain of hPD-1 mAb 9 VL1 (**SEQ ID NO:184**) is shown below (CDR_H residues are shown underlined):

DIQMTQSPSS LSASVGDRVT ITC**RASENIY SYLA**WYQQKP GKAPKLLIY**N**
AKTLAAGVPS RFSGSGSGTD FTLTISSLQP EDFATYYC**QH HYAVPWT**FGQ
GTKLEIK

[00155] An exemplary polynucleotide that encodes hPD-1 mAb 9 VL1 is **SEQ ID NO:185** (nucleotides encoding the CDR_H residues are shown underlined):

gacattcaga tgactcagtc tcccagcagt ctgtccgcat ccgtggggga
tcgggtcacc atcacctgc**c gtgcctcaga aaacatctat tcatacctcg**
cctggtatca acagaaacct ggtaaagccc caaaattgct catttac**aac**
gccaagaccc tcgcagctgg cgtgccaagt aggttctcag gcagcggctc
agggacagat ttcaccctca ccatatcctc actgcagccc gaggattttg
ccacttacta ctgc**cagcat cattacgcag tgccctggac c**ttcggacaa
ggcactaagc tcgagatcaa a

[00156] The amino acid sequence of the VL Domain of hPD-1 mAb 9 VL2 (**SEQ ID NO:186**) is shown below (CDR_H residues are shown underlined):

DIQMTQSPSS LSASVGDRVT ITC**RASENIY NYLA**WYQQKP GKAPKLLIY**D**
AKTLAAGVPS RFSGSGSGTD FTLTISSLQP EDFATYYC**QH HYAVPWT**FGQ
GTKLEIK

[00157] An exemplary polynucleotide that encodes hPD-1 mAb 9 VL2 is **SEQ ID NO:187** (nucleotides encoding the CDR_H residues are shown underlined):

gacattcaga tgactcagtc tcccagcagt ctgtccgcat ccgtggggga
tcgggtcacc atcacctgc**c gtgcctcaga aaacatctat aactacctcg**
cctggtatca acagaaacct ggtaaagccc caaaattgct catttac**gac**
gccaagaccc tcgcagctgg cgtgccaagt aggttctcag gcagcggctc
agggacagat ttcaccctca ccatatcctc actgcagccc gaggattttg
ccacttacta ctgc**cagcat cattacgcag tgccctggac c**ttcggacaa
ggcactaagc tcgagatcaa a

[00158] The CDR_L1 of the VL Domain of hPD-1 mAb 9 VL2 comprises a serine to asparagine amino acid substitution and has the amino acid sequence: RASENIY**N**YLA (**SEQ ID NO:188**), the substituted asparagine is shown underlined). It is contemplated that a similar substitution may be incorporated into any of the PD-1 mAb 9 CDR_L1 Domains described above.

[00159] The CDR_L2 of the VL Domain of hPD-1 mAb 9 VL2 comprises an asparagine to aspartate amino acid substitution and has the amino acid sequence: **D**AKTLAA ((**SEQ ID NO:189**), the substituted aspartate is shown underlined). It is contemplated that a similar substitution may be incorporated into any of the PD-1 mAb 7 CDR_L2 Domains described above.

J. Murine Anti-Human PD-1 Antibody PD-1 mAb 10

[00160] The amino acid sequence of the VH Domain of PD-1 mAb 10 (**SEQ ID NO:190**) is shown below (CDR_H residues are shown underlined).

EVILVESGGG LVKPGGSLKL SCAASGFTFS **NYLMS**WVRQT PEKRLEWVA**S**
ISGGGSNIYY PDSVKGRFTI SRDNAKNTLY LQMNSLRSED TALYYCAR**QE**
LAFDYWGQGT TLTVSS

CDR_H1 of PD-1 mAb 10 (**SEQ ID NO:192**): **NYLMS**

CDR_H2 of PD-1 mAb 10 (**SEQ ID NO:193**): **SISGGGSNIYYPDSVKG**

CDR_H3 of PD-1 mAb 10 (**SEQ ID NO:194**): **QELAFDY**

[00161] An exemplary polynucleotide that encodes the VH Domain of PD-1 mAb 10 is **SEQ ID NO:191** (nucleotides encoding the CDR_H residues are shown underlined):

gaagtgatac tggtggagtc tgggggaggc ttagtgaagc ctggagggtc
cctgaaactc tcctgtgcag cctctggatt cactttcagt **aactatctca**
tgtcttgggt tcgccagact ccggagaaga ggctggagtg ggtcgca**agt**
attagtggtg gtggtagtaa tatctactat ccagacagtg tgaagggtcg
attcaccata tccagggaca atgccaagaa caccctgtac ctgcaaatga
acagtctgag gtctgaggac acggccttgt attactgtgc aaga**caagaa**
ctggcttttg actactgggg ccaaggcacc actctcacag tctcctcc

[00162] The amino acid sequence of the VL Domain of PD-1 mAb 10 (**SEQ ID NO:195**) is shown below (CDR_L residues are shown underlined):

DIQMTQTTSS LSASLGDRVT ISC**RTSQDIS NFLN**WYQQKP DGTIKLLIY**Y**
TSRLHSGVPS RFSGSGSGTD YSLTISNLEQ EDIATYFC**QQ GSTLPWT**FGG
GTKLEII

CDR_L1 of PD-1 mAb 10 (**SEQ ID NO:197**): **RTSQDISNFLN**

CDR_L2 of PD-1 mAb 10 (**SEQ ID NO:198**): **YTSRLHS**

CDR_L3 of PD-1 mAb 10 (**SEQ ID NO:199**): **QQGSTLPWT**

[00163] An exemplary polynucleotide that encodes the VL Domain of PD-1 mAb 10 is **SEQ ID NO:196** (nucleotides encoding the CDR_L residues are shown underlined):

gatatccaga tgacacagac tacatcctcc ctgtctgcct ctctgggaga
cagagtcacc atcagttgc**a ggacaagtca ggacattagc aatttttaa**
actggtatca gcagaaacca gatggaacta ttaaactcct gatctac**tac**
acatcaagat tacactcagg agtcccatca aggttcagtg gcagtgggtc
tggaacagat tattctctca ccattagcaa cctggagcaa gaagatattg
ccacttactt ttgc**caacag ggtagtacgc ttccgtggac g**ttcggtgga
ggcaccaagc tggaaatcat a

K. Murine Anti-Human PD-1 Antibody PD-1 mAb 11

[00164] The amino acid sequence of the VH Domain of PD-1 mAb 11 (**SEQ ID NO:200**) is shown below (CDR_H residues are shown underlined).

EVQLQQSGTV LARPGASVKM SCKTSGYTFT **GYWMH**WVKQR PGQGLKWMG**A**
IYPGNSDTHY NQKFKGKAKL TAVTSASTAY MELSSLTNED SAIYYCTT**GT**
YSYFDVWGTG TTVTVSS

CDR_H1 of PD-1 mAb 11 **(SEQ ID NO:202)**: **GYWMH**

CDR_H2 of PD-1 mAb 11 (**SEQ ID NO:203**): **AIYPGNSDTHYNQKFKG**

CDR_H3 of PD-1 mAb 11 (**SEQ ID NO:204**): **GTYSYFDV**

[00165] An exemplary polynucleotide that encodes the VH Domain of PD-1 mAb 11 is **SEQ ID NO:201** (nucleotides encoding the CDR_H residues are shown underlined):

gaggttcagc tccagcagtc tgggactgtg ctggcaaggc ctggggcttc
agtgaagatg tcctgcaaga cttctggcta cacatttacc ggctactgga
tgcactgggt aaaacagagg cctggacagg gtctgaaatg gatggggget
atttatcctg gaaatagtga tactcactac aaccagaagt tcaagggcaa
ggccaaactg actgcagtca catccgccag cactgcctac atggagctca
gcagcctgac aaatgaggac tctgcgatct attactgtac tactgggacc
tactcgtact tcgatgtctg gggcacaggg accacggtca ccgtctcctc a

[00166] The amino acid sequence of the VL Domain of PD-1 mAb 11 (**SEQ ID NO:205**) is shown below (CDR_L residues are shown underlined):

DILLTQSPAI LSVSPGERVS FSCRASQSIG TSIHWYQHRT NGSPRLLIKY
ASESISGIPS RFSGSGSGTD FTLSINSVES EDIADYYCQQ SNSWLTFGAG
TKLELK

CDR_L1 of PD-1 mAb 11 (**SEQ ID NO:207**): **RASQSIGTSIH**

CDR_L2 of PD-1 mAb 11 (**SEQ ID NO:208**): **YASESIS**

CDR_L3 of PD-1 mAb 11 (**SEQ ID NO:209**): **QQSNSWLT**

[00167] An exemplary polynucleotide that encodes the VL Domain of PD-1 mAb 11 is **SEQ ID NO:206** (nucleotides encoding the CDR_L residues are shown underlined):

gacatcttgc tgactcagtc tccagccatc ctgtctgtga gtccaggaga
aagagtcagt ttctcctgca gggccagtca gagcattggc acaagcatac
actggtatca gcacagaaca aatggttctc caaggcttct cataaagtat
gcttctgagt ctatctctgg gatcccttcc aggtttagtg gcagtggatc
agggactgat tttactctta gcatcaacag tgtggagtct gaagatattg
cagattatta ctgtcaacaa agtaatagct ggctcacgtt cggtgctggg
accaagctgg agctgaaa

L. Murine Anti-Human PD-1 Antibody PD-1 mAb 12

[00168] The amino acid sequence of the VH Domain of PD-1 mAb 12 (**SEQ ID NO:210**) is shown below (CDR_H residues are shown underlined).

QGHLQQSGAE LVRPGASVTL SCKASGFTFT DYEMHWVKQT PVHGLEWIGT
IDPETGGTAY NQKFKGKAIL TVDKSSTTTY MELRSLTSED SAVFYCSRER
ITTVVEGAYW YFDVWGTGTT VTVSS

CDR_H1 of PD-1 mAb 12 **(SEQ ID NO:212)**: **DYEMH**

CDR_H2 of PD-1 mAb 12 (**SEQ ID NO:213**): **TIDPETGGTAYNQKFKG**

CDR_H3 of PD-1 mAb 12 (**SEQ ID NO:214**): **ERITTVVEGAYWYFDV**

[00169] An exemplary polynucleotide that encodes the VH Domain of PD-1 mAb 12 is **SEQ ID NO:211** (nucleotides encoding the CDR_H residues are shown underlined):

cagggtcacc tgcagcagtc tggggctgag ctggtgaggc ctggggcttc
agtgacgctg tcctgcaagg cttcgggctt cacatttact **gactatgaga**
tgcactgggt gaaacagaca cctgtgcatg gcctggaatg gattggg**act**
attgatcctg aaactggtgg tactgcctac aatcagaagt tcaagggcaa
ggccatactg acagtagaca aatcttccac tacaacctac atggagctcc
gcagcctgac atctgaggac tctgccgtct tttattgttc aaga**gagagg**
attactacgg ttgttgaggg ggcatactgg tacttcgatg tctggggcac
agggaccacg gtcaccgtct cctca

[00170] The amino acid sequence of the VL Domain of PD-1 mAb 4 (**SEQ ID NO:215**) is shown below (CDR_L residues are shown underlined):

DVLMTQTPLS LPVSLGDQAS ISC**RSSQNIV HSNGNTYLE**W YLQKPGQSPK
LLIC**KVSTRF S**GVPDRFSGS GSGTDFTLKI SRVEAEDLGV YYC**FQGSHVP**
YTFGGGTKLE IK

CDR_L1 of PD-1 mAb 12 (**SEQ ID NO:217**): **RSSQNIVHSNGNTYLE**

CDR_L2 of PD-1 mAb 12 (**SEQ ID NO:218**): **KVSTRFS**

CDR_L3 of PD-1 mAb 12 (**SEQ ID NO:219**): **FQGSHVPYT**

[00171] An exemplary polynucleotide that encodes the VL Domain of PD-1 mAb 12 is **SEQ ID NO:216** (nucleotides encoding the CDR_L residues are shown underlined):

gatgttttga tgacccagac tccactctcc ctgcctgtca gtcttggaga
tcaagcctcc atctcttgc**a gatctagtca gaacattgta catagtaatg**
gaaacaccta tttagaatgg tacctgcaga aaccaggcca gtctccaaag
ctcctgatct gc**aaagtttc cacccgattt tct**ggggtcc cagacaggtt
cagtggcagt ggatcaggga cagatttcac actcaagatc agcagagtgg
aggctgagga tctgggagtt tattattgc**t ttcaaggttc acatgttccg**
tacacgttcg gagggggggac caagctggaa ataaaa

M. Murine Anti-Human PD-1 Antibody PD-1 mAb 13

[00172] The amino acid sequence of the VH Domain of PD-1 mAb 13 (**SEQ ID NO:220**) is shown below (CDR_H residues are shown underlined).

EVMLVESGGG LVKPGGSLKL SCAASGFTFS **SHTMS**WVRQT PEKRLEWVA**T**
ISGGGSNIYY PDSVKGRFTI SRDNAKNTLY LQMSSLRSED TALYYCAR**QA**
YYGNYWYFDV WGTGTTVTVS S

CDR_H1 of PD-1 mAb 13 **(SEQ ID NO:222)**: **SHTMS**

CDR_H2 of PD-1 mAb 13 (**SEQ ID NO:223**): **TISGGGSNIYYPDSVKG**

CDR$_H$3 of PD-1 mAb 13 (**SEQ ID NO:224**): QAYYGNYWYFDV

[00173] An exemplary polynucleotide that encodes the VH Domain of PD-1 mAb 13 is **SEQ ID NO:221** (nucleotides encoding the CDR$_H$ residues are shown underlined):

gaagtgatgc tggtggagtc tgggggaggc ttagtgaagc ctggagggtc
cctgaaactc tcctgtgcag cctctggatt cactttcagt agccatacca
tgtcttgggt tcgccagact ccggagaaga ggctggagtg ggtcgcaacc
attagtggtg gtggttctaa tatctactat ccagacagtg tgaagggtcg
attcaccatc tccagagaca atgccaagaa caccctgtac ctgcaaatga
gcagtctgag gtctgaggac acggccttgt attactgtgc aagacaagct
tactacggta attactggta cttcgatgtc tggggcacag ggaccacggt
caccgtctcc tcc

[00174] The amino acid sequence of the VL Domain of PD-1 mAb 13 (**SEQ ID NO:225**) is shown below (CDR$_L$ residues are shown underlined):

DIQMTQSPAT QSASLGESVT ITCLASQTIG TWLAWYQQKP GKSPQLLIYA
ATSLADGVPS RFSGSGSGTK FSFKISSLQA EDFVSYYCQQ LDSIPWTFGG
GTKLEIK

CDR$_L$1 of PD-1 mAb 13 (**SEQ ID NO:227**): LASQTIGTWLA

CDR$_L$2 of PD-1 mAb 13 (**SEQ ID NO:228**): AATSLAD

CDR$_L$3 of PD-1 mAb 13 (**SEQ ID NO:229**): QQLDSIPWT

[00175] An exemplary polynucleotide that encodes the VL Domain of PD-1 mAb 13 is **SEQ ID NO:226** (nucleotides encoding the CDR$_L$ residues are shown underlined):

gacattcaga tgacccagtc tcctgccacc cagtctgcat ctctgggaga
aagtgtcacc atcacgtgcc tggcaagtca gaccattggt acatggttag
catggtatca gcagaaacca gggaaatctc ctcagctcct gatttatgct
gcaaccagct tggcagatgg ggtcccatca aggttcagtg gtagtggatc
tggcacaaaa ttttctttca agatcagcag cctacaggct gaagattttg
taagttatta ctgtcaacaa cttgacagta ttccgtggac gttcggtgga
ggcaccaagc tggaaatcaa a

N. Murine Anti-Human PD-1 Antibody PD-1 mAb 14

[00176] The amino acid sequence of the VH Domain of PD-1 mAb 14 (**SEQ ID NO:230**) is shown below (CDR$_H$ residues are shown underlined).

QVQLQQPGAE LVKPGASVKM SCKASGYNFI SYWITWVKQR PGQGLQWIGN
IYPGTDGTTY NEKFKSKATL TVDTSSSTAY MHLSRLTSED SAVYYCATGL
HWYFDVWGTG TTVTVSS

CDR$_H$1 of PD-1 mAb 14 (**SEQ ID NO:232**): SYWIT

CDR$_H$2 of PD-1 mAb 14 (**SEQ ID NO:233**): NIYPGTDGTTYNEKFKS

CDR$_H$3 of PD-1 mAb 14 (**SEQ ID NO:234**): GLHWYFDV

[00177] An exemplary polynucleotide that encodes the VH Domain of PD-1 mAb 14 is **SEQ ID NO:231** (nucleotides encoding the CDR$_H$ residues are shown underlined):

caggtccaac tgcagcagcc tggggctgag cttgtgaagc ctggggcttc
agtgaagatg tcctgcaagg cttctggcta caacttcatc agctactgga
taacctgggt gaaacagagg cctggacaag gccttcagtg gattggaaat
atttatcctg gtactgatgg tactacctac aatgagaagt tcaagagcaa
ggccacactg actgtagaca catcctccag cacagcctac atgcacctca
gtcgcctgac atctgaggac tctgcggtct attactgtgc aactgggcta
cactggtact tcgatgtctg gggcacaggg accacggtca ccgtctcctc c

[00178] The amino acid sequence of the VL Domain of PD-1 mAb 14 (**SEQ ID NO:235**) is shown below (CDR$_L$ residues are shown underlined):

DIVMTQSQKF MSTSVGDRVS VTCKASQSVG TNVAWYQQKP GQSPKALIYS
ASSRFSGVPD RFTGSGSGTD FTLTISNVQS EDLAEYFCQQ YNSYPYTFGG
GTKLEIK

CDR$_L$1 of PD-1 mAb 14 (**SEQ ID NO:237**): KASQSVGTNVA

CDR$_L$2 of PD-1 mAb 14 (**SEQ ID NO:238**): SASSRFS

CDR$_L$3 of PD-1 mAb 14 (**SEQ ID NO:239**): QQYNSYPYT

[00179] An exemplary polynucleotide that encodes the VL Domain of PD-1 mAb 14 is **SEQ ID NO:236** (nucleotides encoding the CDR$_L$ residues are shown underlined):

gacattgtga tgacccagtc tcaaaaattc atgtccacat cagtaggaga
cagggtcagt gtcacctgca aggccagtca gagtgtgggt actaatgtag
cctggtatca acagaagccc ggtcaatctc ctaaagcact gatttactcg
gcatcctccc gattcagtgg cgtccctgat cgcttcacag gcagtggatc
tgggacagat ttcactctca ccatcagtaa tgtgcagtct gaagacttgg
cagagtattt ctgtcagcaa tataacagct atccgtacac gttcggaggg
gggaccaagc tggaaataaa a

O. The Anti-Human PD-1 Antibody PD-1 mAb 15

1. Murine Anti-Human PD-1 Antibody PD-1 mAb 15

[00180] The amino acid sequence of the VH Domain of PD-1 mAb 15 (**SEQ ID NO:240**) is shown below (CDR$_H$ residues are shown underlined).

EVMLVESGGG LVKPGGSLKL SCAASGFIFS SYLISWVRQT PEKRLEWVAA
ISGGGADTYY ADSVKGRFTI SRDNAKNTLY LQMSSLRSED TALYYCTRRG
TYAMDYWGQG TSVTVSS

CDR_H1 of PD-1 mAb 15 **(SEQ ID NO:242)**: SYLIS

CDR_H2 of PD-1 mAb 15 **(SEQ ID NO:243)**: AISGGGADTYYADSVKG

CDR_H3 of PD-1 mAb 15 **(SEQ ID NO:244)**: RGTYAMDY

[00181] An exemplary polynucleotide that encodes the VH Domain of PD-1 mAb 15 is **SEQ ID NO:241** (nucleotides encoding the CDR_H residues are shown underlined):

gaagtgatgc tggtggagtc tgggggaggc ttagtgaagc ctggagggtc
cctgaaactc tcctgtgcag cctctggatt cattttcagt agctatctca
tctcttgggt tcgccagact ccggagaaga ggctggagtg ggtcgctgcc
attagtggtg gtggtgctga cacctactat gccgacagtg tgaagggtcg
attcaccatc tccagagaca atgccaagaa caccctgtat ctgcaaatga
gcagtctgag gtctgaggac acggccttat attactgtac aagacgaggg
acctatgcta tggactactg gggtcaagga acctcagtca ccgtctcctc c

[00182] The amino acid sequence of the VL Domain of PD-1 mAb 15 (**SEQ ID NO:245**) is shown below (CDR_L residues are shown underlined):

DIQMTQSPAS QSASLGESVT ITCLASQTIG TWLAWYQQKP GKSPQLLIYA
ATSLADGVPS RFSGSGSGTK FSFKISSLQA EDFVNYYCQQ LYSIPWTFGG
GTKLEIK

CDR_L1 of PD-1 mAb 15 (**SEQ ID NO:247**): LASQTIGTWLA

CDR_L2 of PD-1 mAb 15 (**SEQ ID NO:248**): AATSLAD

CDR_L3 of PD-1 mAb 15 (**SEQ ID NO:249**): QQLYSIPWT

[00183] An exemplary polynucleotide that encodes the VL Domain of PD-1 mAb 15 is **SEQ ID NO:246** (nucleotides encoding the CDR_L residues are shown underlined):

gacattcaga tgacccagtc tcccgcctcc cagtctgcat ctctgggaga
aagtgtcacc atcacatgcc tggcaagtca gaccattggt acatggttag
catggtatca gcagaaacca gggaaatctc ctcagctcct gatttatgct
gcaaccagct tggcagatgg ggtcccatca aggttcagtg gtagtggatc
tggcacaaaa ttttctttca agatcagcag cctacaggct gaagattttg
taaattatta ctgtcaacaa ctttacagta ttccgtggac gttcggtgga
ggcaccaagc tggaaatcaa a

2. Humanization of the Anti-Human PD-1 Antibody PD-1 mAb 15 to Form "hPD-1 mAb 15"

[00184] The above-described murine anti-human PD-1 antibody PD-1 mAb 15 was humanized and further deimmunized when antigenic epitopes were identified in order to demonstrate the capability of humanizing an anti-human PD-1 antibody so as to decrease its antigenicity upon administration to a human recipient. The humanization yielded one

humanized VH Domain, designated herein as "**hPD-1 mAb 2 VH1**," and one humanized VL Domains designated herein as "**hPD-1 mAb 1 VL1**." An antibody comprising the humanized VL Domain paired with the humanized VH Domain is referred to as "**hPD-1 mAb 15**."

[00185] The amino acid sequence of the VH Domain of hPD-1 mAb 15 VH1 (**SEQ ID NO:250**) is shown below (CDR_H residues are shown underlined):

EVQLVESGGG LVRPGGSLRL SCAASGFTFS **SYLIS**WVRQA PGKGLEWVA**A**
ISGGGADTYY ADSVKGRFTI SRDNAKNSLY LQMNSLRAED TATYYCAR**RG**
TYAMDYWGQG TLVTVSS

[00186] An exemplary polynucleotide that encodes hPD-1 mAb 15 VH1 is **SEQ ID NO:251** (nucleotides encoding the CDR_H residues are shown underlined):

gaagtgcaac tggttgaaag tggcggcggg ctggtgcggc caggtggttc
actcagactg tcttgtgcag cttcaggctt tacattctcc **tcttatctta**
tctcttgggt gcgccaagcc ccaggtaagg gccttgaatg ggtc**gccgcc**
attagtgggg gtggtgccga tacatattat gccgacagcg tcaagggacg
tttcaccatc agcagggaca acgccaagaa tagcctttac ctgcagatga
actcacttag agctgaagac accgctactt attactgtgc ccgg**cgcggg**
acttacgcta tggactattg gggccagggc accttggtca ctgtctcatc c

[00187] The amino acid sequence of the VH Domain of hPD-1 mAb 15 VL1 (**SEQ ID NO:252**) is shown below (CDR_H residues are shown underlined):

DIQMTQSPSS LSASVGDRVT ITC**LASQTIG TWLA**WYQQKP GKAPKLLIY**A**
ATSLADGVPS RFSGSGSGTD FTFTISSLQP EDFATYYC**QQ LYSIPWT**FGQ
GTKLEIK

[00188] An exemplary polynucleotide that encodes hPD-1 mAb 15 VL1 is **SEQ ID NO:253** (nucleotides encoding the CDR_H residues are shown underlined):

gatatccaga tgacccagtc tcccagctct ctcagtgcaa gcgtaggcga
ccgtgtgacc atcacctgt**c tggccagtca gaccattgga acctggctcg**
cctggtatca gcagaaacct ggcaaggccc ctaagctgct gatttacgcc
gccacctccc tcgcagatgg agtgccctcc cgatttagcg ggtccgggtc
cggcaccgac ttcacattca caatcagcag cctccagccc gaggatttcg
ctacatacta ctgt**caacag ctctactcca ttccatggac c**tttggtcag
ggtactaaac tggagatcaa a

V. Anti-Human PD-1 Antibodies PD-1 mAb 1-15, and Their Derivatives Having an Engineered Fc Region

[00189] In traditional immune function, the interaction of antibody-antigen complexes with cells of the immune system results in a wide array of responses, ranging from effector functions such as antibody dependent cytotoxicity, mast cell degranulation, and phagocytosis to

immunomodulatory signals such as regulating lymphocyte proliferation and antibody secretion. All of these interactions are initiated through the binding of the Fc Region of antibodies or immune complexes to specialized cell surface receptors on hematopoietic cells. The diversity of cellular responses triggered by antibodies and immune complexes results from the structural heterogeneity of the three Fc receptors: FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16). FcγRI (CD64), FcγRIIA (CD32A) and FcγRIII (CD16) are activating (*i.e.*, immune system enhancing) receptors; FcγRIIB (CD32B) is an inhibiting (*i.e.*, immune system dampening) receptor. In addition, interaction with the neonatal Fc Receptor (FcRn) mediates the recycling of IgG molecules from the endosome to the cell surface and release into the blood. The amino acid sequence of exemplary wild-type IgG1 (**SEQ ID NO:1**), IgG2 (**SEQ ID NO:2**), IgG3 (**SEQ ID NO:3**), and IgG4 (**SEQ ID NO:4**) are presented above.

[00190] Modification of the Fc Region normally leads to an altered phenotype, for example altered serum half-life, altered stability, altered susceptibility to cellular enzymes or altered effector function. It may be desirable to modify an antibody or other binding molecule of the present invention with respect to effector function, for example, so as to enhance the effectiveness of such molecule in treating cancer. Reduction or elimination of effector function is desirable in certain cases, for example in the case of antibodies whose mechanism of action involves blocking or antagonism, but not killing of the cells bearing a target antigen. Increased effector function is generally desirable when directed to undesirable cells, such as tumor and foreign cells, where the FcγRs are expressed at low levels, for example, tumor-specific B cells with low levels of FcγRIIB (*e.g.*, non-Hodgkins lymphoma, CLL, and Burkitt's lymphoma). In said embodiments, molecules of the invention with conferred or altered effector function activity are useful for the treatment and/or prevention of a disease, disorder or infection where an enhanced efficacy of effector function activity is desired.

[00191] In certain embodiments, the PD-1-binding molecules of the present invention comprise an Fc Region that possesses one or more modifications (*e.g.*, substitutions, deletions, or insertions) to the sequence of amino acids of a wild-type Fc Region (*e.g.*, **SEQ ID NO:1**), which reduce the affinity and avidity of the Fc Region and, thus, the molecule of the invention, for one or more FcγR receptors. In other embodiments, the molecules of the invention comprise an Fc Region that possesses one or more modifications to the amino acids of the wild-type Fc Region, which increase the affinity and avidity of the Fc Region and, thus, the molecule of the invention, for one or more FcγR receptors. In other embodiments, the molecules

comprise a variant Fc Region wherein said variant confers or mediates increased antibody dependent cell mediated cytotoxicity (ADCC) activity and/or an increased binding to FcγRIIA, relative to a molecule comprising no Fc Region or comprising a wild-type Fc Region. In alternate embodiments, the molecules comprise a variant Fc Region wherein said variant confers or mediates decreased ADCC activity (or other effector function) and/or an increased binding to FcγRIIB, relative to a molecule comprising no Fc Region or comprising a wild-type Fc Region. In some embodiments, the invention encompasses PD-1-binding molecules comprising a variant Fc Region, which variant Fc Region does not show a detectable binding to any FcγR, relative to a comparable molecule comprising the wild-type Fc Region. In other embodiments, the invention encompasses PD-1-binding molecules comprising a variant Fc Region, which variant Fc Region only binds a single FcγR, preferably one of FcγRIIA, FcγRIIB, or FcγRIIIA. Any such increased affinity and/or avidity is preferably assessed by measuring *in vitro* the extent of detectable binding to the FcγR or FcγR-related activity in cells that express low levels of the FcγR when binding activity of the parent molecule (without the modified Fc Region) cannot be detected in the cells, or in cells which express non-FcγR receptor target antigens at a density of 30,000 to 20,000 molecules/cell, at a density of 20,000 to 10,000 molecules/cell, at a density of 10,000 to 5,000 molecules/cell, at a density of 5,000 to 1,000 molecules/cell, at a density of 1,000 to 200 molecules/cell or at a density of 200 molecules/cell or less (but at least 10, 50, 100 or 150 molecules/cell).

[00192] The PD-1-binding molecules of the present invention may comprise a variant Fc Region having altered affinities for an activating and/or inhibitory Fcγ receptor. In one embodiment, the PD-1-binding molecule comprises a variant Fc Region that has increased affinity for FcγRIIB and decreased affinity for FcγRIIIA and/or FcγRIIA, relative to a comparable molecule with a wild-type Fc Region. In another embodiment, the PD-1-binding molecule of the present invention comprise a variant Fc Region, which has decreased affinity for FcγRIIB and increased affinity for FcγRIIIA and/or FcγRIIA, relative to a comparable molecule with a wild-type Fc Region. In yet another embodiment, the PD-1-binding molecules of the present invention comprise a variant Fc Region that has decreased affinity for FcγRIIB and decreased affinity for FcγRIIIA and/or FcγRIIA, relative to a comparable molecule with a wild-type Fc Region. In still another embodiment, the PD-1-binding molecules of the present invention comprise a variant Fc Region, which has unchanged affinity for FcγRIIB and decreased (or increased) affinity for FcγRIIIA and/or FcγRIIA, relative to a comparable molecule with a wild-type Fc Region.

[00193] In certain embodiments, the PD-1-binding molecules of the present invention comprise a variant Fc Region having an altered affinity for FcγRIIIA and/or FcγRIIA such that the immunoglobulin has an enhanced effector function. Non-limiting examples of effector cell functions include antibody dependent cell mediated cytotoxicity, antibody dependent phagocytosis, phagocytosis, opsonization, opsonophagocytosis, cell binding, rosetting, C1q binding, and complement dependent cell mediated cytotoxicity.

[00194] In a preferred embodiment, the alteration in affinity or effector function is at least 2-fold, preferably at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, at least 10-fold, at least 50-fold, or at least 100-fold, relative to a comparable molecule comprising a wild-type Fc Region. In other embodiments of the invention, the variant Fc Region immunospecifically binds one or more FcRs with at least 65%, preferably at least 70%, 75%, 80%, 85%, 90%, 95%, 100%, 125%, 150%, 175%, 200%, 225%, or 250% greater affinity relative to a molecule comprising a wild-type Fc Region. Such measurements can be in vivo or in vitro assays, and in a preferred embodiment are in vitro assays such as ELISA or surface plasmon resonance assays.

[00195] In different embodiments, the PD-1-binding molecules of the present invention comprise a variant Fc Region wherein said variant agonizes at least one activity of an FcγR receptor, or antagonizes at least one activity of an FcγR receptor. In a preferred embodiment, the molecules comprise a variant that antagonizes one or more activities of FcγRIIB, for example, B-cell receptor-mediated signaling, activation of B-cells, B-cell proliferation, antibody production, intracellular calcium influx of B cells, cell cycle progression, FcγRIIB-mediated inhibition of FcεRI signaling, phosphorylation of FcγRIIB, SHIP recruitment, SHIP phosphorylation and association with Shc, or activity of one or more downstream molecules (*e.g.*, MAP kinase, JNK, p38, or Akt) in the FcγRIIB signal transduction pathway. In another embodiment, the PD-1-binding molecules of the present invention comprise a variant that agonizes one or more activities of FcεRI, for example, mast cell activation, calcium mobilization, degranulation, cytokine production, or serotonin release.

[00196] In certain embodiments, the molecules comprise an Fc Region comprising regions from two or more IgG isotypes (*e.g.*, IgG1, IgG2, IgG3 and IgG4). As used herein, an Fc Region is said to be of a particular IgG isotype if its amino acid sequence is most homologous to that isotype relative to other IgG isotypes. The various IgG isotypes exhibit differing physical and functional properties including serum half-life, complement fixation, FcγR

binding affinities and effector function activities (*e.g.*, ADCC, CDC, *etc.*) due to differences in the amino acid sequences of their hinge and/or Fc Regions, for example as described in Flesch and Neppert (1999) J. Clin. Lab. Anal. 14:141-156; Chappel *et al.* (1993) J. Biol. Chem. 33:25124-25131; Chappel *et al.* (1991) Proc. Natl. Acad. Sci. (U.S.A.) 88:9036-9040; or Brüggemann *et al.* (1987) J. Exp. Med 166:1351-1361. This type of variant Fc Region may be used alone, or in combination with an amino acid modification, to affect Fc-mediated effector function and/or binding activity. In combination, the amino acid modification and IgG hinge/Fc Region may display similar functionality (*e.g.*, increased affinity for FcγRIIA) and may act additively or, more preferably, synergistically to modify the effector functionality in the molecule of the invention, relative to a molecule of the invention comprising a wild-type Fc Region. In other embodiments, the amino acid modification and IgG Fc Region may display opposite functionality (*e.g.*, increased and decreased affinity for FcγRIIA, respectively) and may act to selectively temper or reduce a specific functionality in the molecule of the invention, relative to a molecule of the invention not comprising an Fc Region or comprising a wild-type Fc Region of the same isotype.

[00197] In a preferred specific embodiment, the PD-1-binding molecules of the present invention comprise a variant Fc Region, wherein said variant Fc Region comprises at least one amino acid modification relative to a wild-type Fc Region, such that said molecule has an altered affinity for an FcR, provided that said variant Fc Region does not have a substitution at positions that make a direct contact with FcγR based on crystallographic and structural analysis of Fc-FcR interactions such as those disclosed by Sondermann *et al.* (2000) Nature 406:267-73. Examples of positions within the Fc Region that make a direct contact with FcγR are amino acid residues 234-239, amino acid residues 265-269 (B/C loop), amino acid residues 297-299 (C'/E loop), and amino acid residues 327-332 (F/G loop). In some embodiments, the molecules of the invention comprise variant Fc Regions comprise modification of at least one residue that does not make a direct contact with an FcγR based on structural and crystallographic analysis, *e.g.*, is not within the Fc-FcγR binding site.

[00198] Variant Fc Regions are well known in the art, and any known variant Fc Region may be used in the present invention to confer or modify the effector function exhibited by a molecule of the invention comprising an Fc Region (or portion thereof) as functionally assayed, *e.g.*, in an NK dependent or macrophage dependent assay. For example, Fc Region variants identified as altering effector function are disclosed in PCT Publications No. WO 04/063351;

WO 06/088494; WO 07/024249; WO 06/113665; WO 07/021841; WO 07/106707; and WO 2008/140603, and any suitable variant disclosed therein may be used in the present molecules.

[00199] In certain embodiments, the PD-1-binding molecules of the present invention comprise a variant Fc Region, having one or more amino acid modifications in one or more regions, which modification(s) alter (relative to a wild-type Fc Region) the **Ratio of Affinities** of the variant Fc Region to an activating FcγR (such as FcγRIIA or FcγRIIIA) relative to an inhibiting FcγR (such as FcγRIIB):

$$\text{Ratio of Affinities} = \frac{\text{Wild-Type to Variant Change in Affinity to Fc}\gamma\text{R}_{\text{Activating}}}{\text{Wild-Type to Variant Change in Affinity to Fc}\gamma\text{R}_{\text{Inhibiting}}}$$

[00200] Particularly preferred are PD-1-binding molecules of the present invention that possess a variant Fc Region (relative to the wild-type Fc Region) in which the variant Fc Region has a Ratio of Affinities greater than 1. Such molecules have particular use in providing a therapeutic or prophylactic treatment of a disease, disorder, or infection, or the amelioration of a symptom thereof, where an enhanced efficacy of effector cell function (*e.g.*, ADCC) mediated by FcγR is desired, *e.g.*, cancer or infectious disease. In contrast, a variant Fc Region having a Ratio of Affinities less than 1 mediates decreased efficacy of effector cell function. **Table 1** lists exemplary single, double, triple, quadruple and quintuple mutations by whether their Ratio of Affinities is greater than or less than 1.

Table 1 Exemplary Single and Multiple Mutations Listed by Ratio of Affinities				
Single	**Double**	**Triple**	**Quadruple**	**Quintuple**
Ratio of Affinities > 1				
F243L	F243L & R292P	F243L, P247L & N421K	L234F, F243L, R292P & Y300L	L235V, F243L, R292P, Y300L & P396L
D270E	F243L & Y300L	F243L, R292P & Y300L	L235I, F243L, R292P & Y300L	L235P, F243L, R292P, Y300L & P396L
R292G	F243L & P396L	F243L, R292P & V305I	L235Q, F243L, R292P & Y300L	F243L, R292P, V305I, Y300L & P396L
R292P	D270E & P396L	F243L, R292P & P396L	F243L, P247L, D270E & N421K	
	R292P & Y300L	F243L, Y300L & P396L	F243L, R255L, D270E & P396L	
	R292P & V305I	P247L, D270E & N421K	F243L, D270E, G316D & R416G	
	R292P & P396L	R255L, D270E & P396L	F243L, D270E, K392T & P396L	
	Y300L & P396L	D270E, G316D & R416G	F243L, D270E, P396L & Q419H	
	P396L & Q419H	D270E, K392T & P396L	F243L, R292P, Y300L, & P396L	
		D270E, P396L & Q419H	F243L, R292P, V305I & P396L	
		V284M, R292L & K370N	P247L, D270E, Y300L & N421K	
		R292P, Y300L & P396L	R255L, D270E, R292G & P396L	
			R255L, D270E, Y300L & P396L	
			D270E, G316D, P396L & R416G	
Ratio of Affinities < 1				
Y300L	F243L & P396L	F243L, R292P & V305I		
P396L	P247L & N421K			
	R255L & P396L			
	R292P & V305I			
	K392T & P396L			
	P396L & Q419H			

[00201] In a specific embodiment, in variant Fc Regions, any amino acid modifications (*e.g.*, substitutions) at any of positions 235, 240, 241, 243, 244, 247, 262, 263, 269, 298, 328, or 330

and preferably one or more of the following residues: A240, I240, L241, L243, H244, N298, I328 or V330. In a different specific embodiment, in variant Fc Regions, any amino acid modifications (*e.g.*, substitutions) at any of positions 268, 269, 270, 272, 276, 278, 283, 285, 286, 289, 292, 293, 301, 303, 305, 307, 309, 331, 333, 334, 335, 337, 338, 340, 360, 373, 376, 416, 419, 430, 434, 435, 437, 438 or 439 and preferably one or more of the following residues: H280, Q280, Y280, G290, S290, T290, Y290, N294, K295, P296, D298, N298, P298, V298, I300 or L300.

[00202] In a preferred embodiment, in variant Fc Regions that bind an FcγR with an altered affinity, any amino acid modifications (*e.g.*, substitutions) at any of positions 255, 256, 258, 267, 268, 269, 270, 272, 276, 278, 280, 283, 285, 286, 289, 290, 292, 293, 294, 295, 296, 298, 300, 301, 303, 305, 307, 309, 312, 320, 322, 326, 329, 330, 332, 331, 333, 334, 335, 337, 338, 339, 340, 359, 360, 373, 376, 416, 419, 430, 434, 435, 437, 438 or 439. Preferably, the variant Fc Region has any of the following residues: A256, N268, Q272, D286, Q286, S286, A290, S290, A298, M301, A312, E320, M320, Q320, R320, E322, A326, D326, E326, N326, S326, K330, T339, A333, A334, E334, H334, L334, M334, Q334, V334, K335, Q335, A359, A360 or A430.

[00203] In a different embodiment, in variant Fc Regions that bind an FcγR (via its Fc Region) with a reduced affinity, any amino acid modifications (*e.g.*, substitutions) at any of positions 252, 254, 265, 268, 269, 270, 278, 289, 292, 293, 294, 295, 296, 298, 300, 301, 303, 322, 324, 327, 329, 333, 335, 338, 340, 373, 376, 382, 388, 389, 414, 416, 419, 434, 435, 437, 438 or 439.

[00204] In a different embodiment, in variant Fc Regions that bind an FcγR (via its Fc Region) with an enhanced affinity, any amino acid modifications (*e.g.*, substitutions) at any of positions 280, 283, 285, 286, 290, 294, 295, 298, 300, 301, 305, 307, 309, 312, 315, 331, 333, 334, 337, 340, 360, 378, 398 or 430. In a different embodiment, in variant Fc Regions that binds FcγRIIA with an enhanced affinity, any of the following residues: A255, A256, A258, A267, A268, N268, A272, Q272, A276, A280, A283, A285, A286, D286, Q286, S286, A290, S290, M301, E320, M320, Q320, R320, E322, A326, D326, E326, S326, K330, A331, Q335, A337 or A430.

[00205] Preferred variants include one or more modifications at any of positions: 228, 230, 231, 232, 233, 234, 235, 239, 240, 241, 243, 244, 245, 247, 262, 263, 264, 265, 266, 271, 273, 275, 281, 284, 291, 296, 297, 298, 299, 302, 304, 305, 313, 323, 325, 326, 328, 330 or 332.

[00206] Particularly preferred variants include one or more modifications selected from groups A-AI:

A	228E, 228K, 228Y or 228G;
B	230A, 230E, 230Y or 230G;
C	231E, 231K, 231Y, 231P or 231G;
D	232E, 232K, 232Y, 232G;
E	233D;
F	234I or 234F;
G	235D, 235Q, 235P, 235I or 235V;
H	239D, 239E, 239N or 239Q;
I	240A, 240I, 240M or 240T;
J	243R, 243, 243Y, 243L, 243Q, 243W, 243H or 243I;
K	244H;
L	245A;
M	247G, 247V or 247L;
N	262A, 262E, 262I, 262T, 262E or 262F;
O	263A, 263I, 263M or 263T;
P	264F, 264E, 264R, 264I, 264A, 264T or 264W;
Q	265F, 265Y, 265H, 265I, 265L, 265T, 265V, 265N or 265Q;
R	266A, 266I, 266M or 266T;
S	271D, 271E, 271N, 271Q, 271K, 271R, 271S, 271T, 271H, 271A, 271V, 271L, 271I, 271F, 271M, 271Y, 271W or 271G;
T	273I;
U	275L or 275W;
V	281D, 281K, 281Y or 281P;
W	284E, 284N, 284T, 284L, 284Y or284M;
X	291D, 291E, 291Q, 291T, 291H, 291I or 291G;
Y	299A, 299D, 299E, 299F, 299G, 299H, 299I, 299K, 299L, 299M, 299N, 299P, 299Q, 299R, 299S, 299V, 299W or 299Y;
Z	302I;
AA	304D, 304N, 304T, 304H or 304L
AB	305I;
AC	313F;
AD	323I;
AE	325A, 325D, 325E, 325G, 325H, 325I, 325L, 325K, 325R, 325S, 325F, 325M, 325T, 325V, 325Y, 325W or 325P;
AF	328D, 328Q, 328K, 328R, 328S, 328T, 328V, 328I, 328Y, 328W, 328P, 328G, 328A, 328E, 328F, 328H, 328M or 328N;
AG	330L, 330Y, 330I or 330V;
AH	332A, 332D, 332E, 332H, 332N, 332Q, 332T, 332K, 332R, 332S, 332V, 332L, 332F, 332M, 332W, 332P, 332G or 332Y; and
AI	336E, 336K or 336Y

[00207] Still more particularly preferred variants include one or more modifications selected from Groups 1-105:

Group	Variant	Group	Variant
1	A330L / I332E	54	S239D / D265L / N297D / I332E
2	D265F / N297E / I332E	55	S239D / D265T / N297D / I332E
3	D265Y / N297D / I332E	56	S239D / D265V / N297D / I332E
4	D265Y / N297D / T299L / I332E	57	S239D / D265Y / N297D / I332E
5	F241E / F243Q / V262T / V264F	58	S239D / I332D
6	F241E / F243Q / V262T / V264E / I332E	59	S239D / I332E
7	F241E / F243R / V262E / V264R	60	S239D / I332E / A330I
8	F241E / F243R / V262E / V264R / I332E	61	S239D / I332N
9	F241E / F243Y / V262T / V264R	62	S239D / I332Q
10	F241E / F243Y / V262T / V264R / I332E	63	S239D / N297D / I332E
11	F241L / F243L / V262I / V264I	64	S239D / N297D / I332E / A330Y
12	F241L / V262I	65	S239D / N297D / I332E / A330Y / F241S / F243H / V262T / V264T
13	F241R / F243Q / V262T / V264R	66	S239D / N297D / I332E / K326E
14	F241R / F243Q / V262T / V264R / I332E	67	S239D / N297D / I332E / L235D
15	F241W / F243W / V262A / V264A	68	S239D / S298A / I332E
16	F241Y / F243Y / V262T / V264T	69	S239D / V264I / A330L / I332E
17	F241Y / F243Y / V262T / V264T / N297D / I332E	70	S239D / V264I / I332E
18	F243L / V262I / V264W	71	S239D / V264I / S298A / I332E
19	P243L / V264I	72	S239E / D265N
20	L328D / I332E	73	S239E / D265Q
21	L328E / I332E	74	S239E / I332D
22	L328H / I332E	75	S239E / I332E
23	L328I / I332E	76	S239E / I332N
24	L328M / I332E	77	S239E / I332Q
25	L328N / I332E	78	S239E / N297D / I332E
26	L328Q / I332E	79	S239E / V264I / A330Y / I332 E
27	L328T / I332E	80	S239E / V264I / I332 E
28	L328V / I332E	81	S239E / V264I / S298A / A330Y / I332E
29	N297D / A330Y / I332E	82	S239N / A330L / I332E
30	N297D / I332E	83	S239N / A330Y / I332E
31	N297D / I332E / S239D / A330L	84	S239N / I332D
32	N297D / S298A / A330Y / I 332E	85	S239N / I332E
33	N297D / T299L / I332E	86	S239N / I332N
34	N297D / T299F / I332E / N297D / T299H / I332E	87	S239N / I332Q
35	N297D / T299I / I332E	88	S239N1S298A / I332E

Group	Variant	Group	Variant
36	N297D / T299L / I332E	89	S239Q / I332D
37	N297D / T299V / I332E	90	S239Q / I332E
38	N297E / I332E	91	S239Q / I332N
39	N297S / I332E	92	S239Q / I332Q
40	P230A / E233D / I332E	93	S239Q / V264I / I332E
41	P244H / P245A / P247V	94	S298A / I332E
42	S239D / A330L / I332E	95	V264E / N297D / I332E
43	S239D / A330Y / I332E	96	V264I / A330L / I332E
44	S239D / A330Y / I332E / K326E	97	V264I / A330Y / I332E
45	S239D / A330Y / I332E / K326T	98	V264I / I332E
46	S239D / A330Y / I332E / L234I	99	V264I / S298A / I332E
47	S239D / A330Y / I332E / L235D	100	Y296D / N297D / I332E
48	S239D / A330Y / I332E / V240I	101	Y296E / N297D / I332 E
49	S239D / A330Y / I332E / V264T	102	Y296H / N297D / I332E
50	S239D / A330Y / I332E / V266I	103	Y296N / N297D / I332E
51	S239D / D265F / N297D / I332E	104	Y296Q / N297I / I332E
52	S239D / D265H / N297D / I332E	105	Y296T / N297D / I332E
53	S239D / D265I / N297D / I332E		

[00208] In one embodiment, a PD-1-binding molecule of the invention will comprise a variant Fc Region having at least one modification in the Fc Region. In certain embodiments, the variant Fc Region comprises at least one substitution selected from the group consisting of L235V, F243L, R292P, Y300L, V305I, and P396L.

[00209] In a specific embodiment, the variant Fc Region comprises:

(A) at least one substitution selected from the group consisting of F243L, R292P, Y300L, V305I, and P396L;

(B) at least two substitutions selected from the group consisting of:

(1) F243L and P396L;

(2) F243L and R292P; and

(3) R292P and V305I;

(C) at least three substitutions selected from the group consisting of:

(1) F243L, R292P and Y300L;

(2) F243L, R292P and V305I;

(3) F243L, R292P and P396L; and

(4) R292P, V305I and P396L;

(D) at least four substitutions selected from the group consisting of:

(1) F243L, R292P, Y300L and P396L; and

(2) F243L, R292P, V305I and P396L; or

(E) at least the five substitutions selected from the group consisting of:
(1) F243L, R292P, Y300L, V305I and P396L; and
(2) L235V, F243L, R292P, Y300L and P396L.

[00210] In another specific embodiment, the variant Fc Region comprises substitutions of:

(A) F243L, R292P, and Y300L;
(B) L235V, F243L, R292P, Y300L, and P396L; or
(C) F243L, R292P, Y300L, V305I, and P396L.

[00211] In one embodiment, a PD-1-binding molecule of the invention comprises a variant Fc Region that exhibits decreased (or substantially no) binding to FcγRIA (CD64), FcγRIIA (CD32A), FcγRIIB (CD32B), FcγRIIIA (CD16a) or FcγRIIIB (CD16b) (relative to the binding exhibited by the wild-type IgG1 Fc Region (**SEQ ID NO:1**)). In one embodiment, a PD-1-binding molecule of the invention will comprise a variant Fc Region that exhibits reduced (or substantially no) binding to an FcγR (*e.g.*, FcγRIIIA) and reduced (or substantially no) ADCC effector function. In certain embodiments, the variant Fc Region comprises at least one substitution selected from the group consisting of L234A, L235A, D265A,N297Q, and N297G. In a specific embodiment, the variant Fc Region comprises the substitution of L234A; L235A; L234A and L235A; D265A; N297Q, or N297G.

[00212] A preferred IgG1 sequence for the CH2 and CH3 Domains of the PD-1-binding molecules of the invention will have the L234A/L235A substitutions (**SEQ ID NO:5**):

APE**AA**GGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD
GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE
WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE
ALHNHYTQKS LSLSPG**X**

wherein, **X** is a lysine (K) or is absent.

[00213] In a different embodiment, a PD-1-binding molecule of the invention comprises an Fc Region which inherently exhibits decreased (or substantially no) binding to FcγRIIIA (CD16a) and/or reduced effector function (relative to the binding exhibited by the wild-type IgG1 Fc Region (**SEQ ID NO:1**)). In a specific embodiment, a PD-1-binding molecule of the present invention comprises an IgG2 Fc Region (**SEQ ID NO:2**) or an IgG4 Fc Region (**SEQ ID:NO:4**). When an IgG4 Fc Region in utilized, the instant invention also encompasses the introduction of a stabilizing mutation, such the IgG4 hinge region S228P substitution (see, *e.g.*,

SEQ ID NO:13: ESKYGPPCPPCP, (Lu *et al.*, (2008) "*The Effect Of A Point Mutation On The Stability Of Igg4 As Monitored By Analytical Ultracentrifugation*," J. Pharmaceutical Sciences 97:960-969) to reduce the incidence of strand exchange. Other stabilizing mutations known in the art may be introduced into an IgG4 Fc Region (Peters, P *et al.*, (2012) "*Engineering an Improved IgG4 Molecule with Reduced Disulfide Bond Heterogeneity and Increased Fab Domain Thermal Stability*," J. Biol. Chem., 287:24525-24533; PCT Patent Publication No: WO 2008/145142).

[00214] In other embodiments, the invention encompasses the use of any variant Fc Region known in the art, such as those disclosed in Jefferis, B.J. *et al.* (2002) "*Interaction Sites On Human IgG-*Fc *For FcgammaR: Current Models*," Immunol. Lett. 82:57-65; Presta, L.G. *et al.* (2002) "*Engineering Therapeutic Antibodies For Improved Function*," Biochem. Soc. Trans. 30:487-90; Idusogie, E.E. *et al.* (2001) "*Engineered Antibodies With Increased Activity To Recruit Complement*," J. Immunol. 166:2571-75; Shields, R.L. *et al.* (2001) "*High Resolution Mapping Of The Binding Site On Human IgG1 For Fc Gamma RI, Fc Gamma RII, Fc Gamma RIII, And FcRn And Design Of IgG1 Variants With Improved Binding To The Fc gamma R*," J. Biol. Chem. 276:6591-6604; Idusogie, E.E. *et al.* (2000) "*Mapping Of The C1q Binding Site On Rituxan, A Chimeric Antibody With A Human IgG Fc*," J. Immunol. 164:4178-84; Reddy, M.P. *et al.* (2000) "*Elimination Of Fc Receptor-Dependent Effector Functions Of A Modified IgG4 Monoclonal Antibody To Human CD4*," J. Immunol. 164:1925-1933; Xu, D. *et al.* (2000) "*In Vitro Characterization of Five Humanized OKT3 Effector Function Variant Antibodies*," Cell. Immunol. 200:16-26; Armour, K.L. *et al.* (1999) "*Recombinant human IgG Molecules Lacking Fcgamma Receptor I Binding And Monocyte Triggering Activities*," Eur. J. Immunol. 29:2613-24; Jefferis, R. *et al.* (1996) "*Modulation Of Fc(Gamma)R And Human Complement Activation By IgG3-Core Oligosaccharide Interactions*," Immunol. Lett. 54:101-04; Lund, J. *et al.* (1996) "*Multiple Interactions Of IgG With Its Core Oligosaccharide Can Modulate Recognition By Complement And Human Fc Gamma Receptor I And Influence The Synthesis Of Its Oligosaccharide Chains*," J. Immunol. 157:4963-4969; Hutchins *et al.* (1995) "*Improved Biodistribution, Tumor Targeting, And Reduced Immunogenicity In Mice With A Gamma 4 Variant Of Campath-1H*," Proc. Natl. Acad. Sci. (U.S.A.) 92:11980-84; Jefferis, R. *et al.* (1995) "*Recognition Sites On Human IgG For Fc Gamma Receptors: The Role Of Glycosylation*," Immunol. Lett. 44:111-17; Lund, J. *et al.* (1995) "*Oligosaccharide-Protein Interactions In IgG Can Modulate Recognition By Fc Gamma Receptors*," FASEB J. 9:115-19; Alegre, M.L. *et al.* (1994) "*A Non-Activating "Humanized" Anti-CD3 Monoclonal*

Antibody Retains Immunosuppressive Properties In Vivo," Transplantation 57:1537-1543; Lund *et al.* (1992) "*Multiple Binding Sites On The CH2 Domain Of IgG For Mouse Fc Gamma R11*," Mol. Immunol. 29:53-59; Lund *et al.* (1991) "*Human Fc Gamma RI And Fc Gamma RII Interact With Distinct But Overlapping Sites On Human IgG*," J. Immunol. 147:2657-2662; Duncan, A.R. *et al.* (1988) "*Localization Of The Binding Site For The Human High-Affinity Fc Receptor On IgG*," Nature 332:563-564; US Patent Nos. 5,624,821; 5,885,573; 6,194,551; 7,276,586; and 7,317,091; and PCT Publications WO 00/42072 and PCT WO 99/58572.

[00215] In some embodiments, the molecules of the invention further comprise one or more glycosylation sites, so that one or more carbohydrate moieties are covalently attached to the molecule. Preferably, the molecules of the invention with one or more glycosylation sites and/or one or more modifications in the Fc Region confer or have an enhanced antibody mediated effector function, *e.g.*, enhanced ADCC activity, compared to the unmodified antibody. In some embodiments, the invention further comprises molecules comprising one or more modifications of amino acids that are directly or indirectly known to interact with a carbohydrate moiety of the Fc Region, including but not limited to amino acids at positions 241, 243, 244, 245, 245, 249, 256, 258, 260, 262, 264, 265, 296, 299, and 301. Amino acids that directly or indirectly interact with a carbohydrate moiety of an Fc Region are known in the art, *see*, *e.g.*, Jefferis *et al.*, 1995 *Immunology Letters*, 44: 111-7, which is incorporated herein by reference in its entirety.

[00216] In another embodiment, the invention encompasses molecules that have been modified by introducing one or more glycosylation sites into one or more sites of the molecules, preferably without altering the functionality of the molecules, *e.g.*, binding activity to target antigen or FcγR. Glycosylation sites may be introduced into the variable and/or constant region of the molecules of the invention. As used herein, "glycosylation sites" include any specific amino acid sequence in an antibody to which an oligosaccharide (*i.e.*, carbohydrates containing two or more simple sugars linked together) will specifically and covalently attach. Oligosaccharide side chains are typically linked to the backbone of an antibody via either N-or O-linkages. N-linked glycosylation refers to the attachment of an oligosaccharide moiety to the side chain of an asparagine residue. O-linked glycosylation refers to the attachment of an oligosaccharide moiety to a hydroxyamino acid, *e.g.*, serine, threonine. The molecules of the invention may comprise one or more glycosylation sites, including N-linked and O-linked glycosylation sites. Any glycosylation site for N-linked or O-linked glycosylation known in

the art may be used in accordance with the instant invention. An exemplary N-linked glycosylation site that is useful in accordance with the methods of the present invention is the amino acid sequence: Asn-X-Thr/Ser, wherein X may be any amino acid and Thr/Ser indicates a threonine or a serine. Such a site or sites may be introduced into a molecule of the invention using methods well known in the art to which this invention pertains (see for example, *IN VITRO* MUTAGENESIS, RECOMBINANT DNA: A SHORT COURSE, J. D. Watson, *et al.* W.H. Freeman and Company, New York, 1983, chapter 8, pp. 106-116, which is incorporated herein by reference in its entirety. An exemplary method for introducing a glycosylation site into a molecule of the invention may comprise: modifying or mutating an amino acid sequence of the molecule so that the desired Asn-X-Thr/Ser sequence is obtained.

[00217] In some embodiments, the invention encompasses methods of modifying the carbohydrate content of a molecule of the invention by adding or deleting a glycosylation site. Methods for modifying the carbohydrate content of antibodies (and molecules comprising antibody domains, *e.g.,* Fc Region) are well known in the art and encompassed within the invention, *see*, *e.g.*, U.S. Patent No. 6,218,149; EP 0 359 096 B1; U.S. Publication No. US 2002/0028486; WO 03/035835; U.S. Publication No. 2003/0115614; U.S. Patent No. 6,218,149; U.S. Patent No. 6,472,511; all of which are incorporated herein by reference in their entirety. In other embodiments, the invention encompasses methods of modifying the carbohydrate content of a molecule of the invention by deleting one or more endogenous carbohydrate moieties of the molecule. In a specific embodiment, the invention encompasses shifting the glycosylation site of the Fc Region of an antibody, by modifying positions adjacent to 297. In a specific embodiment, the invention encompasses modifying position 296 so that position 296 and not position 297 is glycosylated.

[00218] Effector function can also be modified by techniques such as by introducing one or more cysteine residues into the Fc Region, thereby allowing interchain disulfide bond formation in this region to occur, resulting in the generation of a homodimeric antibody that may have improved internalization capability and/or increased complement-mediated cell killing and ADCC (Caron, P.C. et *al.* (1992) "*Engineered Humanized Dimeric Forms Of IgG Are More Effective Antibodies*," J. Exp. Med. 176:1191-1195; Shopes, B. (1992) "*A Genetically Engineered Human IgG Mutant With Enhanced Cytolytic Activity*," J. Immunol. 148(9):2918-2922. Homodimeric antibodies with enhanced antitumor activity may also be prepared using heterobifunctional cross-linkers as described in Wolff, E.A. *et al.* (1993)

"*Monoclonal Antibody Homodimers: Enhanced Antitumor Activity In Nude Mice*," Cancer Research 53:2560-2565. Alternatively, an antibody can be engineered which has dual Fc Regions and may thereby have enhanced complement lysis and ADCC capabilities (Stevenson, G.T. *et al.* (1989) "*A Chimeric Antibody With Dual Fc Regions (bisFabFc) Prepared By Manipulations At The IgG Hinge*," Anti-Cancer Drug Design 3:219-230).

[00219] The serum half-life of the molecules of the present invention comprising Fc Regions may be increased by increasing the binding affinity of the Fc Region for FcRn. The term "half-life" as used herein means a pharmacokinetic property of a molecule that is a measure of the mean survival time of the molecules following their administration. Half-life can be expressed as the time required to eliminate fifty percent (50%) of a known quantity of the molecule from a subject's body (*e.g.*, human patient or other mammal) or a specific compartment thereof, for example, as measured in serum, *i.e.*, circulating half-life, or in other tissues. In general, an increase in half-life results in an increase in mean residence time (MRT) in circulation for the molecule administered.

[00220] In some embodiments, the PD-1-binding molecules of the present invention comprise a variant Fc Region, wherein said variant Fc Region comprises at least one amino acid modification relative to a wild-type Fc Region, such that said molecule has an increased half-life (relative to a wild-type Fc Region).

[00221] In some embodiments, the PD-1-binding molecules of the present invention comprise a variant Fc Region, wherein said variant Fc Region comprises a half-live extending amino acid substitution at one or more positions selected from the group consisting of 238, 250, 252, 254, 256, 257, 256, 265, 272, 286, 288, 303, 305, 307, 308, 309, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424, 428, 433, 434, 435, and 436 . Numerous specific mutations capable of increasing the half-life of an Fc Region-containing molecule are known in the art and include, for example M252Y, S254T, T256E, and combinations thereof. For example, see the mutations described in U.S. Patent Nos. 6,277,375, 7,083,784; 7,217,797, 8,088,376; U.S. Publication Nos. 2002/0147311; 2007/0148164; and International Publication Nos. WO 98/23289; WO 2009/058492; and WO 2010/033279, which are herein incorporated by reference in their entireties. Fc Region-containing molecules with enhanced half-life also include those with substitutions at two or more of Fc Region residues 250, 252, 254, 256, 257, 288, 307, 308, 309, 311, 378, 428, 433, 434, 435 and 436. In particular, two or more

substitutions selected from: T250Q, M252Y, S254T, T256E, K288D, T307Q, V308P, A378V, M428L, N434A, H435K, and Y436I.

[00222] In a specific embodiment, the variant Fc Region comprises substitutions of:

(A) M252Y, S254T and T256E;

(B) M252Y and S254T;

(C) M252Y and T256E;

(D) T250Q and M428L;

(E) T307Q and N434A;

(F) A378V and N434A;

(G) N434A and Y436I;

(H) V308P and N434A; or

(I) K288D and H435K.

[00223] The instant invention further encompasses variant Fc Regions comprising:

(A) one or more mutations which alter effector function and/or FcγR; and

(B) one or more mutations which extend serum half-life.

VI. Bispecific Anti-Human PD-1-Binding Molecules

[00224] One embodiment of the present invention relates to bispecific binding molecules that are capable of binding to a "**first epitope**" and a "**second epitope**," wherein the first epitope is an epitope of human PD-1 and the second epitope is the same or a different epitope of PD-1, or is an epitope of another molecule that is present on the surface of an immune cell (such as a T lymphocyte) and is involved in regulating an immune checkpoint. In one embodiment, the second epitope is an epitope of B7-H3, B7-H4, BTLA, CD3, CD8, CD16, CD27, CD32, CD40, CD40L, CD47, CD64, CD70, CD80, CD86, CD94, CD137, CD137L, CD226, CTLA-4, Galectin-9, GITR, GITRL, HHLA2, ICOS, ICOSL, KIR, LAG-3, LIGHT, MHC class I or II, NKG2a, NKG2d, OX40, OX40L, PD1H, PD-1, PD-L1, PD-L2, PVR, SIRPa, TCR, TIGIT, TIM-3 or VISTA. In one embodiment, the second epitope not an epitope of PD-1. In a specific embodiment, the second epitope is CD137, CTLA-4, LAG-3, OX40, TIGIT, or TIM-3. In certain embodiments, a bispecific molecule comprises more than two epitope binding sites. Such bispecific molecules may bind two or more different epitopes of LAG-3 and at least one epitope of a molecule that is not LAG-3.

[00225] The instant invention encompasses bispecific antibodies capable of simultaneously binding to PD-1 and the second epitope (*e.g.* B7-H3, B7-H4, BTLA, CD40, CD80, CD86, CD137, CTLA-4, ICOS, KIR, LAG-3, MHC class I or II, OX40, PD-L1, TCR, TIM-3, *etc.*). In some embodiments, the bispecific antibody capable of simultaneously binding to PD-1 and the second epitope is produced using any of the methods described in PCT Publication Nos. WO 1998/002463, WO 2005/070966, WO 2006/107786 WO 2007/024715, WO 2007/075270, WO 2006/107617, WO 2007/046893, WO 2007/146968, WO 2008/003103, WO 2008/003116, WO 2008/027236, WO 2008/024188, WO 2009/132876, WO 2009/018386, WO 2010/028797, WO2010028796, WO 2010/028795, WO 2010/108127, WO 2010/136172, WO 2011/086091, WO 2011/133886, WO 2012/009544, WO 2013/003652, WO 2013/070565, WO 2012/162583, WO 2012/156430, WO 2013/174873, and WO 2014/022540, each of which is hereby incorporated herein by reference in its entirety.

A. Bispecific Diabodies Lacking Fc Regions

[00226] One embodiment of the present invention relates to bispecific diabodies that comprise, and most preferably are composed of, a first polypeptide chain and a second polypeptide chain, whose sequences permit the polypeptide chains to covalently bind to each other to form a covalently associated diabody that is capable of simultaneously binding to a first epitope and a second epitope, such epitopes not being identical to one another. Such bispecific diabodies thus comprise "**VL1**" / "**VH1**" domains that are capable of binding to the first epitope and "**VL2**" / "**VH2**" domains that are capable of binding to the second epitope. The notation "**VL1**" and "**VH1**" denote respectively, the Variable Light Chain Domain and Variable Heavy Chain Domain that bind the "first" epitope of such bispecific diabody. Similarly, the notation "**VL2**" and "**VH2**" denote respectively, the Variable Light Chain Domain and Variable Heavy Chain Domain that bind the "second" epitope of such bispecific diabody. It is irrelevant whether a particular epitope is designated as the first vs. the second epitope; such notation having relevance only with respect to the presence and orientation of domains of the polypeptide chains of the binding moleucles of the present invention. In one embodiment, one of such epitopes is an epitope of PD-1 and the other of such epitopes is not an epitope of PD-1 (for example, an epitope of B7-H3, B7-H4, BTLA, CD40, CD80, CD86, CD137, CTLA-4, ICOS, KIR, LAG-3, MHC class I or II, OX40, PD-L1, TCR, TIM-3, *etc.*).

[00227] The VL Domain of the first polypeptide chain interacts with the VH Domain of the second polypeptide chain to form a first functional antigen-binding site that is specific for a

first antigen (*i.e.*, either PD-1 or an antigen that contains the second epitope). Likewise, the VL Domain of the second polypeptide chain interacts with the VH Domain of the first polypeptide chain in order to form a second functional antigen-binding site that is specific for a second antigen (*i.e.*, either an antigen that contains the second epitope or PD-1). Thus, the selection of the VL and VH Domains of the first and second polypeptide chains is coordinated, such that the two polypeptide chains of the diabody collectively comprise VL and VH Domains capable of binding to both an epitope of PD-1 and to the second epitope (*i.e.*, they comprise $VL_{PD\text{-}1}/VH_{PD\text{-}1}$ and VL2/VH2, wherein PD-1 is the "first" epitope, or VL1/VH1 and $VL_{PD\text{-}1}/VH_{PD\text{-}1}$, wherein PD-1 is the "second" epitope).

[00228] The first polypeptide chain of an embodiment of such bispecific diabodies comprises, in the N-terminal to C-terminal direction, an N-terminus, the VL1 Domain of a monoclonal antibody capable of binding to either the first or second epitope (*i.e.*, either $VL_{PD\text{-}1}$ or $VL_{Epitope\ 2}$), a first intervening spacer peptide (Linker 1), a VH2 Domain of a monoclonal antibody capable of binding to either the second epitope (if such first polypeptide chain contains $VL_{PD\text{-}1}$) or the first epitope (if such first polypeptide chain contains $VL_{Epitope\ 2}$), a second intervening spacer peptide (Linker 2) optionally containing a cysteine residue, a Heterodimer-Promoting Domain and a C-terminus (**Figure 1**).

[00229] The second polypeptide chain of this embodiment of bispecific diabodies comprises, in the N-terminal to C-terminal direction, an N-terminus, a VL2 Domain of a monoclonal antibody capable of binding to either PD-1 or the second epitope (*i.e.*, either $VL_{PD\text{-}1}$ or $VL_{Epitope\ 2}$, and being the VL Domain not selected for inclusion in the first polypeptide chain of the diabody), an intervening linker peptide (Linker 1), a VH1 Domain of a monoclonal antibody capable of binding to either the second epitope (if such second polypeptide chain contains $VL_{PD\text{-}1}$) or to PD-1 (if such second polypeptide chain contains $VL_{Epitope\ 2}$), a second intervening spacer peptide (Linker 2) optionally containing a cysteine residue, a Heterodimer-Promoting Domain, and a C-terminus (**Figure 1**).

[00230] Most preferably, the length of the intervening linker peptide (*e.g.*, Linker 1) that separates such VL and VH Domains is selected to substantially or completely prevent the VL and VH Domains of the polypeptide chain from binding to one another. Thus the VL and VH Domains of the first polypeptide chain are substantially or completely incapable of binding to one another. Likewise, the VL and VH Domains of the second polypeptide chain are

substantially or completely incapable of binding to one another. A preferred intervening spacer peptide (Linker 1) has the sequence (**SEQ ID NO:14**): GGGSGGGG.

[00231] The length and composition of the second intervening linker peptide (Linker 2) is selected based on the choice of heterodimer-promoting domains. Typically, the second intervening linker peptide (Linker 2) will comprise 3-20 amino acid residues. In particular, where the heterodimer-promoting domains do not comprise a cysteine residue a cysteine-containing second intervening linker peptide (Linker 2) is utilized. A cysteine-containing second intervening spacer peptide (Linker 2) will contain 1, 2, 3 or more cysteines. A preferred cysteine-containing spacer peptide (Linker 2) has the sequence is **SEQ ID NO:15**: GGCGGG. Alternatively, Linker 2 does not comprise a cysteine (*e.g.*, GGG, GGGS (**SEQ ID NO:29**), LGGGSG (**SEQ ID NO:261**), GGGSGGGSGGG (**SEQ ID NO:262**), ASTKG (**SEQ ID NO:30**), LEPKSS (**SEQ ID NO:33**), APSSS (**SEQ ID NO:34**), *etc.*) and a Cysteine-Containing Heterodimer-Promoting Domain, as described below is used. Optionally, both a cysteine-containing Linker 2 and a cysteine-containing Heterodimer-Promoting Domain are used.

[00232] The Heterodimer-Promoting Domains may be GVEPKSC (**SEQ ID NO:16**) or VEPKSC (**SEQ ID NO:17**) or AEPKSC (**SEQ ID NO:18**) on one polypeptide chain and GFNRGEC (**SEQ ID NO:19**) or FNRGEC (**SEQ ID NO:20**) on the other polypeptide chain (US2007/0004909).

[00233] More preferably, however, the Heterodimer-Promoting Domains of such diabodies are formed from one, two, three or four tandemly repeated coil domains of opposing charge that comprise a sequence of at least six, at least seven or at least eight amino acid residues such that the Heterodimer-Promoting Domain possesses a net charge (Apostolovic, B. *et al.* (2008) "*pH-Sensitivity of the E3/K3 Heterodimeric Coiled Coil*," Biomacromolecules 9:3173–3180; Arndt, K.M. *et al.* (2001) "*Helix-stabilized Fv (hsFv) Antibody Fragments: Substituting the Constant Domains of a Fab Fragment for a Heterodimeric Coiled-coil Domain*," J. Molec. Biol. 312:221-228; Arndt, K.M. *et al.* (2002) "*Comparison of In Vivo Selection and* Rational *Design of Heterodimeric Coiled Coils*," Structure 10:1235-1248; Boucher, C. *et al.* (2010) "*Protein Detection By Western Blot Via Coiled–Coil Interactions*," Analytical Biochemistry 399:138-140; Cachia, P.J. *et al.* (2004) "*Synthetic Peptide Vaccine Development: Measurement Of Polyclonal Antibody Affinity And Cross-Reactivity Using A New Peptide Capture And Release System For Surface Plasmon Resonance Spectroscopy*," J. Mol.

Recognit. 17:540-557; De Crescenzo, G.D. *et al.* (2003) "*Real-Time Monitoring of the Interactions of Two-Stranded de novo Designed Coiled-Coils: Effect of Chain Length on the Kinetic and Thermodynamic Constants of Binding*," Biochemistry 42:1754-1763; Fernandez-Rodriquez, J. *et al.* (2012) "*Induced Heterodimerization And Purification Of Two Target Proteins By A Synthetic Coiled-Coil Tag*," Protein Science 21:511-519; Ghosh, T.S. *et al.* (2009) "*End-To-End And End-To-Middle Interhelical Interactions: New Classes Of Interacting Helix Pairs In Protein Structures*," Acta Crystallographica D65:1032-1041; Grigoryan, G. *et al.* (2008) "*Structural Specificity In Coiled-Coil Interactions*," Curr. Opin. Struc. Biol. 18:477-483; Litowski, J.R. *et al.* (2002) "*Designing Heterodimeric Two-Stranded α-Helical Coiled-Coils: The Effects Of Hydrophobicity And α-Helical Propensity On Protein Folding, Stability, And Specificity*," J. Biol. Chem. 277:37272-37279; Steinkruger, J.D. *et al.* (2012) "*The d'--d--d' Vertical Triad is Less Discriminating Than the a'--a--a' Vertical Triad in the Antiparallel Coiled-coil Dimer Motif*," J. Amer. Chem. Soc. 134(5):2626–2633; Straussman, R. *et al.* (2007) "*Kinking the Coiled Coil – Negatively Charged Residues at the Coiled-coil Interface*," J. Molec. Biol. 366:1232-1242; Tripet, B. *et al.* (2002) "*Kinetic Analysis of the Interactions between Troponin C and the C-terminal Troponin I Regulatory Region and Validation of a New Peptide Delivery/Capture System used for Surface Plasmon Resonance*," J. Molec. Biol. 323:345–362; Woolfson, D.N. (2005) "*The Design Of Coiled-Coil Structures And Assemblies*," Adv. Prot. Chem. 70:79-112; Zeng, Y. *et al.* (2008) "*A Ligand-Pseudoreceptor System Based On de novo Designed Peptides For The Generation Of Adenoviral Vectors With Altered Tropism*," J. Gene Med. 10:355-367).

[00234] Such repeated coil domains may be exact repeats or may have substitutions. For example, the coil domain of the Heterodimer-Promoting Domain of the first polypeptide chain may comprise a sequence of eight amino acid residues selected to confer a negative charge to such Heterodimer-Promoting Domain, and the coil domain of the Heterodimer-Promoting Domain of the second polypeptide chain may comprise a sequence of eight amino acid residues selected to confer a positive charge to such Heterodimer-Promoting Domain. It is immaterial which coil is provided to the first or second polypeptide chains, provided that a coil of opposite charge is used for the other polypeptide chain. The positively charged amino acid may be lysine, arginine, histidine, *etc.* and/or the negatively charged amino acid may be glutamic acid, aspartic acid, *etc.* The positively charged amino acid is preferably lysine and/or the negatively charged amino acid is preferably glutamic acid. It is possible for only a single Heterodimer-Promoting Domain to be employed (since such domain will inhibit homodimerization and

thereby promote heterodimerization), however, it is preferred for both the first and second polypeptide chains of the diabodies of the present invention to contain Heterodimer-Promoting Domains.

[00235] In a preferred embodiment, one of the Heterodimer-Promoting Domains will comprise four tandem "E-coil" helical domains (**SEQ ID NO:21**: EVAALEK-EVAALEK-EVAALEK-EVAALEK), whose glutamate residues will form a negative charge at pH 7, while the other of the Heterodimer-Promoting Domains will comprise four tandem "K-coil" domains (**SEQ ID NO:22**: KVAALKE-KVAALKE-KVAALKE-KVAALKE), whose lysine residues will form a positive charge at pH 7. The presence of such charged domains promotes association between the first and second polypeptides, and thus fosters heterodimer formation. Especially preferred is a Heterodimer-Promoting Domain in which one of the four tandem "E-coil" helical domains of **SEQ ID NO:21** has been modified to contain a cysteine residue: EVAACEK-EVAALEK-EVAALEK-EVAALEK (**SEQ ID NO:23**). Likewise, especially preferred is a Heterodimer-Promoting Domain in which one of the four tandem "K-coil" helical domains of **SEQ ID NO:22** has been modified to contain a cysteine residue: KVAACKE-KVAALKE-KVAALKE-KVAALKE (**SEQ ID NO:24**).

[00236] As disclosed in WO 2012/018687, in order to improve the *in vivo* pharmacokinetic properties of diabodies, a diabody may be modified to contain a polypeptide portion of a serum-binding protein at one or more of the termini of the diabody. Most preferably, such polypeptide portion of a serum-binding protein will be installed at the C-terminus of the diabody. Albumin is the most abundant protein in plasma and has a half-life of 19 days in humans. Albumin possesses several small molecule binding sites that permit it to non-covalently bind to other proteins and thereby extend their serum half-lives. The Albumin-Binding Domain 3 (ABD3) of protein G of Streptococcus strain G148 consists of 46 amino acid residues forming a stable three-helix bundle and has broad albumin-binding specificity (Johansson, M.U. *et al.* (2002) "*Structure, Specificity, And Mode Of Interaction For Bacterial Albumin-Binding Module*s," J. Biol. Chem. 277(10):8114-8120. Thus, a particularly preferred polypeptide portion of a serum-binding protein for improving the *in vivo* pharmacokinetic properties of a diabody is the Albumin-Binding Domain (ABD) from streptococcal protein G, and more preferably, the Albumin-Binding Domain 3 (ABD3) of protein G of *Streptococcus dysgalactiae* strain G148 (**SEQ ID NO:25**): LAEAKVLANR ELDKYGVSDY YKNLIDNAKS AEGVKALIDE ILAALP.

[00237] As disclosed in WO 2012/162068 (herein incorporated by reference), "**deimmunized**" variants of **SEQ ID NO:25** have the ability to attenuate or eliminate MHC class II binding. Based on combinational mutation results, the following combinations of substitutions are considered to be preferred substitutions for forming such a deimmunized ABD: 66D/70S +71A; 66S/70S +71A; 66S/70S +79A; 64A/65A/71A; 64A/65A/71A+66S; 64A/65A/71A+66D; 64A/65A/71A+66E; 64A/65A/79A+66S; 64A/65A/79A+66D; 64A/65A/79A+66E. Variant ABDs having the modifications L64A, I65A and D79A or the modifications N66S, T70S and D79A. Variant deimmunized ABD having the amino acid sequence:

LAEAKVLANR ELDKYGVSDY YKNLI**D**$_{66}$NAK**S**$_{70}$ **A**$_{71}$EGVKALIDE ILAALP (**SEQ ID NO:26**),

or the amino acid sequence:

LAEAKVLANR ELDKYGVSDY YKN**A**$_{64}$**A**$_{65}$NNAKT VEGVKALI**A**$_{79}$E ILAALP (**SEQ ID NO:27**),

or the amino acid sequence:

LAEAKVLANR ELDKYGVSDY YKNLI**S**$_{66}$NAK**S**$_{70}$ VEGVKALI**A**$_{79}$E ILAALP (**SEQ ID NO:28**),

are particularly preferred as such deimmunized ABD exhibit substantially wild-type binding while providing attenuated MHC class II binding. Thus, the first polypeptide chain of such a diabody having an ABD contains a peptide linker preferably positioned C-terminally to the E-coil (or K-coil) Domain of such polypeptide chain so as to intervene between the E-coil (or K-coil) Domain and the ABD (which is preferably a deimmunized ABD). A preferred sequence for such a peptide linker is **SEQ ID NO:29**: GGGS.

B. Bispecific Diabodies Containing Fc Regions

[00238] One embodiment of the present invention relates to bispecific diabodies comprising an Fc Region capable of simultaneously binding to PD-1 and a second epitope (*e.g.* B7-H3, B7-H4, BTLA, CD40, CD80, CD86, CD137, CTLA-4, ICOS, KIR, LAG-3, MHC class I or II, OX40, PD-1, PD-L1, TCR, TIM-3, *etc.*). The addition of an IgG CH2-CH3 Domain to one or both of the diabody polypeptide chains, such that the complexing of the diabody chains results in the formation of an Fc Region, increases the biological half-life and/or alters the valency of the diabody. Incorporating an IgG CH2-CH3 Domains onto both of the diabody polypeptides will permit a two-chain bispecific Fc-Region-containing diabody to form (**Figure 2**).

[00239] Alternatively, incorporating an IgG CH2-CH3 Domains onto only one of the diabody polypeptides will permit a more complex four-chain bispecific Fc Region-containing diabody to form (**Figures 3A-3C**). **Figure 3C** shows a representative four-chain diabody possessing the Constant Light (CL) Domain and the Constant Heavy CH1 Domain, however fragments of such domains as well as other polypeptides may alternatively be employed (see, *e.g.*, **Figures 3A and 3B**, United States Patent Publications No. 2013-0295121; 2010-0174053 and 2009-0060910; European Patent Publication No. EP 2714079; EP 2601216; EP 2376109; EP 2158221 and PCT Publications No. WO 2012/162068; WO 2012/018687; WO 2010/080538). Thus, for example, in lieu of the CH1 Domain, one may employ a peptide having the amino acid sequence GVEPKSC (**SEQ ID NO:16**) VEPKSC (**SEQ ID NO:17**), or AEPKSC (**SEQ ID NO:18**), derived from the hinge domain of a human IgG, and in lieu of the CL Domain, one may employ the C-terminal 6 amino acids of the human kappa light chain, GFNRGEC (**SEQ ID NO:19**) or FNRGEC (**SEQ ID NO:20**). A representative peptide containing four-chain diabody is shown in **Figure 3A**. Alternatively, or in addition, one may employ a peptide comprising tandem coil domains of opposing charge such as the "E-coil" helical domains (**SEQ ID NO:21**: EVAALEK-EVAALEK-EVAALEK-EVAALEK or **SEQ ID NO:23:** EVAACEK-EVAALEK-EVAALEK-EVAALEK); and the "K-coil" domains (**SEQ ID NO:22**: KVAALKE-KVAALKE-KVAALKE-KVAALKE or **SEQ ID NO:24:** KVAACKE-KVAALKE-KVAALKE-KVAALKE). A representative coil domain containing four-chain diabody is shown in **Figure 3B**.

[00240] The Fc Region-containing diabody molecules of the present invention generally include additional intervening linker peptides (Linkers). Typically, the additional Linkers will comprise 3-20 amino acid residues. Additional or alternative linkers that may be employed in the Fc Region-containing diabody molecules of the present invention include: GGGS (**SEQ ID NO:29**), LGGGSG (**SEQ ID NO:261**), GGGSGGGSGGG (**SEQ ID NO:262**), ASTKG (**SEQ ID NO:30**), DKTHTCPPCP (**SEQ ID NO:31**), EPKSCDKTHTCPPCP (**SEQ ID NO:32**), LEPKSS (**SEQ ID NO:33**), APSSS (**SEQ ID NO:34**), and APSSSPME (**SEQ ID NO:35**), LEPKSADKTHTCPPC **SEQ ID NO:36**), GGC, and GGG. **SEQ ID NO:33** may be used in lieu of GGG or GGC for ease of cloning. Additionally, the amino acids GGG, or **SEQ ID NO:33** may be immediately followed by **SEQ ID NO:31** to form the alternate linkers: GGGDKTHTCPPCP (**SEQ ID NO:263**); and LEPKSSDKTHTCPPCP (**SEQ ID NO:37**). Fc Region-containing diabody molecule of the present invention may incorporate an IgG hinge

region in addition to or in place of a linker. Exemplary hinge regions include: EPKSCDKTHTCPPCP (**SEQ ID NO:32**) from IgG1, ERKCCVECPPCP (**SEQ ID NO:11**) from IgG2, ESKYGPPCPSCP (**SEQ ID NO:12**) from IgG4, and ESKYGPPCPPCP (**SEQ ID NO:13**) an IgG4 hinge variant comprising a stabilizing substitute to reduce strand exchange.

[00241] As provided in **Figure 3A-3C**, diabodies of the invention may comprise four different chains. The first and third polypeptide chains of such a diabody contain three domains: (i) a VL1-containing Domain, (ii) a VH2-containing Domain, (iii) Heterodimer-Promoting Domain and (iv) a Domain containing a CH2-CH3 sequence. The second and fourth polypeptide chains contain: (i) a VL2-containing Domain, (ii) a VH1-containing Domain and (iii) a Heterodimer-Promoting Domain, where the Heterodimer-Promoting Domains promote the dimerization of the first/third polypeptide chains with the second/fourth polypeptide chains. The VL and/or VH Domains of the third and fourth polypeptide chains, and VL and/or VH Domains of the first and second polypeptide chains may be the same or different so as to permit tetravalent binding that is either monospecific, bispecific or tetraspecific. The notation "**VL3**" and "**VH3**" denote respectively, the Variable Light Chain Domain and Variable Heavy Chain Domain that bind the "third" epitope of such diabody. Similarly, the notation "**VL4**" and "**VH4**" denote respectively, the Variable Light Chain Domain and Variable Heavy Chain Domain that bind the "fourth" epitope of such diabody. The general structure of the polypeptide chains of a representative four-chain Fc Region-containing diabodies of invention is provided in **Table 2**:

Table 2

Bispecific	2nd Chain	NH_2-VL2-VH1-HPD-COOH
	1st Chain	NH_2-VL1-VH2-HPD-CH2-CH3-COOH
	1st Chain	NH_2-VL1-VH2-HPD-CH2-CH3-COOH
	2nd Chain	NH_2-VL2-VH1-HPD-COOH
Tetraspecific	2nd Chain	NH_2-VL2-VH1-HPD-COOH
	1st Chain	NH_2-VL1-VH2-HPD-CH2-CH3-COOH
	3rd Chain	NH_2-VL3-VH4-HPD-CH2-CH3-COOH
	4th Chain	NH_2-VL4-VH3-HPD-COOH

HPD = Heterodimer-Promoting Domain

[00242] In a specific embodiment, diabodies of the present invention are bispecific, tetravalent (*i.e.*, possess four epitope-binding sites), Fc-containing diabodies (**Figures 3A-3C**)

that are composed of four total polypeptide chains. The bispecific, tetravalent, Fc-containing diabodies of the invention comprise two epitope-binding sites immunospecific for PD-1 (which may be capable of binding to the same epitope of PD-1 or to different epitopes of PD-1), and two epitope-binding sites specific for a second epitope (*e.g.*, B7-H3, B7-H4, BTLA, CD40, CD80, CD86, CD137, CTLA-4, ICOS, KIR, LAG-3 MHC class I or II, OX40, PD-L1, TCR, TIM-3, *etc.*).

[00243] In a further embodiment, the bispecific Fc Region-containing diabodies may comprise three polypeptide chains. The first polypeptide of such a diabody contains three domains: (i) a VL1-containing Domain, (ii) a VH2-containing Domain and (iii) a Domain containing a CH2-CH3 sequence. The second polypeptide of such diabodies contains: (i) a VL2-containing Domain, (ii) a VH1-containing Domain and (iii) a Domain that promotes heterodimerization and covalent bonding with the diabody's first polypeptide chain. The third polypeptide of such diabodies comprises a CH2-CH3 sequence. Thus, the first and second polypeptide chains of such diabodies associate together to form a VL1/VH1 binding site that is capable of binding to the first epitope, as well as a VL2/VH2 binding site that is capable of binding to the second epitope. The first and second polypeptides are bonded to one another through a disulfide bond involving cysteine residues in their respective Third Domains. Notably, the first and third polypeptide chains complex with one another to form an Fc Region that is stabilized via a disulfide bond. Such diabodies have enhanced potency. **Figures 4A** and **4B** illustrate the structures of such diabodies. Such Fc-Region-containing bispecific diabodies may have either of two orientations (**Table 3**):

Table 3

First Orientation	3rd Chain	NH_2-CH2-CH3-COOH
	1st Chain	NH_2-VL1-VH2-HPD-CH2-CH3-COOH
	2nd Chain	NH_2-VL2-VH1-HPD-COOH
Second Orientation	3rd Chain	NH_2-CH2-CH3-COOH
	1st Chain	NH_2-CH2-CH3-VL1-VH2-HPD-COOH
	2nd Chain	NH_2-VL2-VH1-HPD-COOH

HPD = Heterodimer-Promoting Domain

[00244] In a specific embodiment, diabodies of the present invention are bispecific, bivalent (*i.e.*, possess two epitope-binding sites), Fc-containing diabodies (**Figures 4A-4B**) that are composed of three total polypeptide chains. The bispecific, bivalent Fc-containing diabodies

of the invention comprise one epitope-binding site immunospecific for PD-1, and one epitope-binding site specific for a second epitope (*e.g.*, B7-H3, B7-H4, BTLA, CD40, CD80, CD86, CD137, CTLA-4, ICOS, KIR, LAG-3 MHC class I or II, OX40, PD-L1, TCR, TIM-3, *etc.*).

[00245] In a further embodiment, the bispecific Fc Region-containing diabodies may comprise a total of five polypeptide chains. In a particular embodiment, two of said five polypeptide chains have the same amino acid sequence. The first polypeptide chain of such diabodies contains: (i) a VH1-containing domain, (ii) a CH1-containing domain, and (iii) a Domain containing a CH2-CH3 sequence. The first polypeptide chain may be the heavy chain of an antibody that contains a VH1 and a heavy chain constant region. The second and fifth polypeptide chains of such diabodies contain: (i) a VL1-containing domain, and (ii) a CL-containing domain. The second and/or fifth polypeptide chains of such diabodies may be light chains of an antibody that contains a VL1 complementary to the VH1 of the first/third polypeptide chain. The first, second and/or fifth polypeptide chains may be isolated from naturally occurring antibodies. Alternatively, they may be constructed recombinantly. The third polypeptide chain of such diabodies contains: (i) a VH1-containing domain, (ii) a CH1-containing domain, (iii) a Domain containing a CH2-CH3 sequence, (iv) a VL2-containing Domain, (v) a VH3-containing Domain and (vi) a Heterodimer-Promoting Domain, where the Heterodimer-Promoting Domains promote the dimerization of the third chain with the fourth chain. The fourth polypeptide of such diabodies contains: (i) a VL3-containing Domain, (ii) a VH2-containing Domain and (iii) a Domain that promotes heterodimerization and covalent bonding with the diabody's third polypeptide chain.

[00246] Thus, the first and second, and the third and fifth, polypeptide chains of such diabodies associate together to form two VL1/VH1 binding sites capable of binding a first epitope. The third and fourth polypeptide chains of such diabodies associate together to form a VL2/VH2 binding site that is capable of binding to a second epitope, as well as a VL3/VH3 binding site that is capable of binding to a third epitope. The first and third polypeptides are bonded to one another through a disulfide bond involving cysteine residues in their respective constant regions. Notably, the first and third polypeptide chains complex with one another to form an Fc Region. Such diabodies have enhanced potency. **Figure 5** illustrates the structure of such diabodies. It will be understood that the VL1/VH1, VL2/VH2, and VL3/VH3 Domains may be the same or different so as to permit binding that is monospecific, bispecific or trispecific. However, as provided herein, these domains are preferably selected so as to bind

PD-1 and a second epitope (*e.g.*, B7-H3, B7-H4, BTLA, CD40, CD80, CD86, CD137, CTLA-4, ICOS, KIR, LAG-3 MHC class I or II, OX40, PD-L1, TCR, TIM-3, *etc.*).

[00247] The VL and VH Domains of the polypeptide chains are selected so as to form VL/VH binding sites specific for a desired epitope. The VL/VH binding sites formed by the association of the polypeptide chains may be the same or different so as to permit tetravalent binding that is monospecific, bispecific, trispecific or tetraspecific. In particular, the VL and VH Domains maybe selected such that a bispecific diabody may comprise two binding sites for a first epitope and two binding sites for a second epitope, or three binding sites for a first epitope and one binding site for a second epitope, or two binding sites for a first epitope, one binding site for a second epitope and one binding site for a third epitope (as depicted in **Figure 5**). The general structure of the polypeptide chains of representative five-chain Fc Region-containing diabodies of invention is provided in **Table 4**:

Table 4

Bispecific (2x2)	2nd Chain	NH_2-VL1-CL-COOH
	1st Chain	NH_2-VH1-CH1-CH2-CH3-COOH
	3rd Chain	NH_2-VH1-CH1-CH2-CH3-VL2-VH2-HPD-COOH
	5nd Chain	NH_2-VL1-CL-COOH
	4th Chain	NH_2-VL2-VH2-HPD-COOH
Bispecific (3x1)	2nd Chain	NH_2-VL1-CL-COOH
	1st Chain	NH_2-VH1-CH1-CH2-CH3-COOH
	3rd Chain	NH_2-VH1-CH1-CH2-CH3-VL1-VH2-HPD-COOH
	5nd Chain	NH_2-VL1-CL-COOH
	4th Chain	NH_2-VL2-VH1-HPD-COOH
Trispecific (2x1x1)	2nd Chain	NH_2-VL1-CL-COOH
	1st Chain	NH_2-VH1-CH1-CH2-CH3-COOH
	3rd Chain	NH_2-VH1-CH1-CH2-CH3-VL2-VH3-HPD-COOH
	5nd Chain	NH_2-VL1-CL-COOH
	4th Chain	NH_2-VL3-VH2-HPD-COOH

HPD = Heterodimer-Promoting Domain

[00248] In a specific embodiment, diabodies of the present invention are bispecific, tetravalent (*i.e.*, possess four epitope-binding sites), Fc-containing diabodies that are composed of five total polypeptide chains having two binding sites for a first epitope and two binding sites for a second epitope. In one embodiment, the bispecific, tetravalent, Fc-containing

diabodies of the invention comprise two epitope-binding sites immunospecific for PD-1 (which may be capable of binding to the same epitope of PD-1 or to different epitopes of PD-1), and two epitope-binding sites specific for a second epitope (*e.g.*, B7-H3, B7-H4, BTLA, CD40, CD80, CD86, CD137, CTLA-4, ICOS, KIR, LAG-3 MHC class I or II, OX40, PD-L1, TCR, TIM-3, *etc.*). In another embodiment, the bispecific, tetravalent, Fc-containing diabodies of the invention comprise three epitope-binding sites immunospecific for PD-1 which may be capable of binding to the same epitope of PD-1 or to different epitopes of PD-1), and one epitope-binding sites specific for a second epitope (*e.g.*, B7-H3, B7-H4, BTLA, CD40, CD80, CD86, CD137, CTLA-4, ICOS, KIR, LAG-3 MHC class I or II, OX40, PD-L1, TCR, TIM-3, *etc.*). In another embodiment, the bispecific, tetravalent, Fc-containing diabodies of the invention comprise one epitope-binding sites immunospecific for PD-1, and three epitope-binding sites specific for a second epitope (*e.g.*, B7-H3, B7-H4, BTLA, CD40, CD80, CD86, CD137, CTLA-4, ICOS, KIR, LAG-3 MHC class I or II, OX40, PD-L1, TCR, TIM-3, *etc.*).

C. Bispecific Trivalent Binding Molecules Containing Fc Regions

[00249] A further embodiment of the present invention relates to bispecific, trivalent binding molecules, comprising an Fc Region, and being capable of simultaneously binding to a first epitope, a second epitope and a third epitope, wherein at least one of such epitopes is not identical to another. Such bispecific diabodies thus comprise "**VL1**" / "**VH1**" domains that are capable of binding to the first epitope, "**VL2**" / "**VH2**" domains that are capable of binding to the second epitope and "**VL3**" / "**VH3**" domains that are capable of binding to the third epitope. In one embodiment, one or two of such epitopes is an epitope of PD-1 and another (or the other) of such epitopes is not an epitope of PD-1 (for example, an epitope of B7-H3, B7-H4, BTLA, CD40, CD80, CD86, CD137, CTLA-4, ICOS, KIR, LAG-3, MHC class I or II, OX40, PD-1, PD-L1, TCR, TIM-3, *etc.*). Such bispecific trivalent binding molecules comprise three epitope-binding sites, two of which are diabody-type binding domains, which provide binding Site A and binding Site B, and one of which is a non-diabody-type binding domain, which provides binding Site C (see, *e.g.*, **Figures 6A-6F**, and PCT Application No: PCT/US15/33081; and PCT/US15/33076).

[00250] Typically, the trivalent binding molecules of the present invention will comprise four different polypeptide chains (see **Figures 6A-6B**), however, the molecules may comprise fewer or greater numbers of polypeptide chains, for example by fusing such polypeptide chains to one another (*e.g.*, via a peptide bond) or by dividing such polypeptide chains to form

additional polypeptide chains, or by associating fewer or additional polypeptide chains via disulfide bonds. **Figures 6B-6F** illustrate this aspect of the present invention by schematically depicting such molecules having three polypeptide chains. As provided in **Figures 6A-6F**, the trivalent binding molecules of the present invention may have alternative orientations in which the diabody-type binding domains are N-terminal (**Figures 6A**, **6C** and **6D**) or C-terminal (**Figures 6B**, **6E** and **6F**) to an Fc Region.

[00251] In certain embodiments, the first polypeptide chain of such trivalent binding molecules of the present invention contains: (i) a **VL1-containing Domain**, (ii) a **VH2**-containing Domain, (iii) a Heterodimer-Promoting Domain, and (iv) a Domain containing a CH2-CH3 sequence. The VL1 and VL2 Domains are located N-terminal or C-terminal to the CH2-CH3-containing domain as presented in **Table 5 (Figures 6A and 6B)**. The second polypeptide chain of such embodiments contains: (i) a VL2-containing Domain, (ii) a VH1-containing Domain, and (iii) a Heterodimer-Promoting Domain. The third polypeptide chain of such embodiments contains: (i) a VH3-containing Domain, (ii) a CH1-containing Domain and (iii) a Domain containing a CH2-CH3 sequence. The third polypeptide chain may be the heavy chain of an antibody that contains a VH3 and a heavy chain constant region. The fourth polypeptide of such embodiments contains: (i) a VL3-containing Domain and (ii) a CL-containing Domain. The fourth polypeptide chains may be a light chain of an antibody that contains a VL3 complementary to the VH3 of the third polypeptide chain. The third or fourth polypeptide chains may be isolated from naturally occurring antibodies. Alternatively, they may be constructed recombinantly, synthetically or by other means.

[00252] The Variable Light Chain Domain of the first and second polypeptide chains are separated from the Variable Heavy Chain Domains of such polypeptide chains by an intervening spacer linker having a length that is too short to permit their VL1/VH2 (or their VL2/VH1) domains to associate together to form epitope-binding site capable of binding to either the first or second epitope. A preferred intervening spacer peptide (Linker 1) for this purpose has the sequence (**SEQ ID NO:14**): GGGSGGGG. Other Domains of the trivalent binding molecules may be separated by one or more intervening spacer peptides, optionally comprising a cysteine residue. Exemplary linkers useful for the generation of trivalent binding molecules are provided herein and are also provided in PCT Application Nos: PCT/US15/33081; and PCT/US15/33076. Thus, the first and second polypeptide chains of such trivalent binding molecules associate together to form a VL1/VH1 binding site capable of

binding a first epitope, as well as a VL2/VH2 binding site that is capable of binding to a second epitope. The third and fourth polypeptide chains of such trivalent binding molecules associate together to form a VL3/VH3 binding site that is capable of binding to a third epitope. It will be understood that the VL1/VH1, VL2/VH2, and VL3/VH3 Domains may be the same or different so as to permit binding that is monospecific, bispecific or trispecific.

[00253] As described above, the trivalent binding molecules of the present invention may comprise three polypeptides. Trivalent binding molecules comprising three polypeptide chains may be obtained by linking the domains of the fourth polypeptide N-terminal to the VH3-containing Domain of the third polypeptide. Alternatively, a third polypeptide chain of a trivalent binding molecule of the invention containing the following three domains is utilized: (i) a VL3-containing Domain, (ii) a VH3-containing Domain, and (iii) a Domain containing a CH2-CH3 sequence, wherein the VL3 and VH3 are spaced apart from one another by an intervening spacer peptide that is sufficiently long (at least 9 or more amino acid residues) so as to allow the association of these domains to form an epitope-binding site.

[00254] It will be understood that the VL1/VH1, VL2/VH2, and VL3/VH3 Domains may be the same or different so as to permit binding that is monospecific, bispecific or trispecific. However, as provided herein, these domains are preferably selected so as to bind PD-1 and a second epitope (or a second and third epitope) (preferably, such epitopes are epitopes of B7-H3, B7-H4, BTLA, CD40, CD80, CD86, CD137, CTLA-4, ICOS, KIR, LAG-3 MHC class I or II, OX40, PD-L1, TCR, TIM-3, *etc.*).

[00255] In particular, the VL and VH Domains may be selected such that a trivalent binding molecule comprises two binding sites for a first epitope and one binding sites for a second epitope, or one binding site for a first epitope and two binding sites for a second epitope, or one binding site for a first epitope, one binding site for a second epitope and one binding site for a third epitope. The general structure of the polypeptide chains of representative trivalent binding molecules of invention is provided in **Figures 6A-6F** and in **Table 5**:

Table 5		
Four Chain 1st Orientation	2nd Chain	NH_2-VL2-VH1-HPD-COOH
	1st Chain	NH_2-VL1-VH2-HPD-CH2-CH3-COOH
	3rd Chain	NH_2-VH3-CH1-CH2-CH3-COOH
	4th Chain	NH_2-VL3-CL-COOH
Four Chain 2nd Orientation	2nd Chain	NH_2-VL2-VH1-HPD-COOH
	1st Chain	NH_2 -CH2-CH3--VL1-VH2-HPD COOH
	3rd Chain	NH_2-VH3-CH1-CH2-CH3-COOH
	4th Chain	NH_2-VL3-CL-COOH
Three Chain 1st Orientation	2nd Chain	NH_2-VL2-VH1-HPD-COOH
	1st Chain	NH_2-VL1-VH2-HPD-CH2-CH3-COOH
	3rd Chain	NH_2-VL3-VH3-HPD-CH2-CH3-COOH
Three Chain 2nd Orientation	2nd Chain	NH_2-VL2-VH1-HPD-COOH
	1st Chain	NH_2 -CH2-CH3-VL1-VH2-HPD COOH
	3rd Chain	NH_2-VL3-VH3-HPD-CH2-CH3-COOH

HPD = Heterodimer-Promoting Domain

[00256] One embodiment of the present invention relates to bispecific trivalent binding molecules that comprise two epitope-binding sites for PD-1 and one epitope-binding site for the second epitope present on a molecule other than PD-1 (*e.g.* B7-H3, B7-H4, BTLA, CD40, CD80, CD86, CD137, CTLA-4, ICOS, KIR, LAG-3, MHC class I or II, OX40, PD-L1, TCR, TIM-3, *etc.*). The two epitope-binding sites for PD-1 may bind the same epitope or different epitopes. Another embodiment of the present invention relates to bispecific trivalent binding molecules that comprise, one epitope-binding site for PD-1 and two epitope-binding sites that bind a second antigen present on a molecule other than PD-1 (*e.g.* B7-H3, B7-H4, BTLA, CD40, CD80, CD86, CD137, CTLA-4, ICOS, KIR, LAG-3, MHC class I or II, OX40, PD-L1, TCR, TIM-3, *etc.*). The two epitope-binding sites for the second antigen may bind the same epitope or different epitopes of the antigen (*e.g.*, the same or different epitopes of LAG-3). As provided above, such bispecific trivalent binding molecules may comprise three or four polypeptide chains.

VII. Constant Domains and Fc Regions

[00257] Provided herein are antibody Constant Domains useful in the generation of the PD-1-binding molecules (*e.g.*, antibodies, diabodies, trivalent binding molecules, *etc.*) of the invention.

[00258] A preferred CL Domain is a human IgG CL Kappa Domain. The amino acid sequence of an exemplary human CL Kappa Domain is (**SEQ ID NO:8**):

RTVAAPSVFI FPPSDEQLKS GTASVVCLLN NFYPREAKVQ WKVDNALQSG
NSQESVTEQD SKDSTYSLSS TLTLSKADYE KHKVYACEVT HQGLSSPVTK
SFNRGEC

[00259] Alternatively, an exemplary CL Domain is a human IgG CL Lambda Domain. The amino acid sequence of an exemplary human CL Kappa Domain is (**SEQ ID NO:9**):

QPKAAPSVTL FPPSSEELQA NKATLVCLIS DFYPGAVTVA WKADSSPVKA
GVETTPSKQS NNKYAASSYL SLTPEQWKSH RSYSCQVTHE GSTVEKTVAP
TECS

[00260] As provided herein, the PD-1-binding molecules of the invention may comprise an Fc Region. The Fc Region of such molecules of the invention may be of any isotype (*e.g.*, IgG1, IgG2, IgG3, or IgG4). The PD-1-binding molecules of the invention may further comprise a CH1 Domain and/or a hinge region. When present, the CH1 Domain and/or hinge region may be of any isotype (*e.g.*, IgG1, IgG2, IgG3, or IgG4), and is preferably of the same isotype as the desired Fc Region.

[00261] An exemplary CH1 Domain is a human IgG1 CH1 Domain. The amino acid sequence of an exemplary human IgG1 CH1 Domain is (**SEQ ID NO:10**):

ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV
HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKRV

[00262] An exemplary CH1 Domain is a human IgG2 CH1 Domain. The amino acid sequence of an exemplary human IgG2 CH1 Domain is (**SEQ ID NO:257**):

ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV
HTFPAVLQSS GLYSLSSVVT VPSSNFGTQT YTCNVDHKPS NTKVDKTV

[00263] An exemplary CH1 Domain is a human IgG4 CH1 Domain. The amino acid sequence of an exemplary human IgG4 CH1 Domain is (**SEQ ID NO:254**):

ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV
HTFPAVLQSS GLYSLSSVVT VPSSSLGTKT YTCNVDHKPS NTKVDKRV

[00264] One exemplary hinge region is a human IgG1 hinge region. The amino acid sequence of an exemplary human IgG1 hinge region is (**SEQ ID NO:32**): EPKSCDKTHTCPPCP.

[00265] Another exemplary hinge region is a human IgG2 hinge region. The amino acid sequence of an exemplary human IgG2 hinge region is (**SEQ ID NO:11**): ERKCCVECPPCP.

[00266] Another exemplary hinge region is a human IgG4 hinge region. The amino acid sequence of an exemplary human IgG4 hinge region is (**SEQ ID NO:12**): ESKYGPPCPSCP. As described herein, an IgG4 hinge region may comprise a stabilizing mutation such as the S228P substitution. The amino acid sequence of an exemplary stabilized IgG4 hinge region is (**SEQ ID NO:13**): ESKYGPPCPPCP.

[00267] The Fc Region of the Fc Region-containing molecules (*e.g.*, antibodies, diabodies, and trivalent molecules) of the present invention may be either a complete Fc Region (*e.g.*, a complete IgG Fc Region) or only a fragment of an Fc Region. Optionally, the Fc Region of the Fc Region-containing molecules of the present invention lacks the C-terminal lysine amino acid residue. In particular, the Fc Region of the Fc Region-containing molecules of the present invention may be an engineered variant Fc Region. Although the Fc Region of the bispecific Fc Region-containing molecules of the present invention may possess the ability to bind to one or more Fc receptors (*e.g.*, FcγR(s)), more preferably such variant Fc Region have altered binding to FcγRIA (CD64), FcγRIIA (CD32A), FcγRIIB (CD32B), FcγRIIIA (CD16a) or FcγRIIIB (CD16b) (relative to the binding exhibited by a wild-type Fc Region) or will have substantially reduced or no ability to bind to inhibitory receptor(s). Thus, the Fc Region of the Fc Region-containing molecules of the present invention may include some or all of the CH2 Domain and/or some or all of the CH3 Domain of a complete Fc Region, or may comprise a variant CH2 and/or a variant CH3 sequence (that may include, for example, one or more insertions and/or one or more deletions with respect to the CH2 or CH3 domains of a complete Fc Region). Such Fc Regions may comprise non-Fc polypeptide portions, or may comprise portions of non-naturally complete Fc Regions, or may comprise non-naturally occurring orientations of CH2 and/or CH3 Domains (such as, for example, two CH2 domains or two CH3 domains, or in the N-terminal to C-terminal direction, a CH3 Domain linked to a CH2 Domain, *etc.*).

[00268] Fc Region modifications identified as altering effector function are known in the art, including modifications that increase binding to activating receptors (*e.g.*, FcγRIIA (CD16A)

and reduce binding to inhibitory receptors (*e.g.*, FcγRIIB (CD32B) (see, *e.g.*, Stavenhagen, J.B. *et al.* (2007) "*Fc Optimization Of Therapeutic Antibodies Enhances Their Ability To Kill Tumor Cells In Vitro And Controls Tumor Expansion In Vivo Via Low-Affinity Activating Fcgamma Receptors*," Cancer Res. 57(18):8882-8890). Exemplary variants of human IgG1 Fc Regions with reduced binding to CD32B and/or increased binding to CD16A contain F243L, R292P, Y300L, V305I or P296L substitutions. These amino acid substitutions may be present in a human IgG1 Fc Region in any combination or sub-combination. In one embodiment, the human IgG1 Fc Region variant contains a F243L, R292P and Y300L substitution. In another embodiment, the human IgG1 Fc Region variant contains F243L, R292P, Y300L, V305I and P296L substitutions.

[00269] In particular, it is preferred for the Fc Regions of the polypeptide chains of the Fc Region-containing molecules of the present invention to exhibit decreased (or substantially no) binding to FcγRIA (CD64), FcγRIIA (CD32A), FcγRIIB (CD32B), FcγRIIIA (CD16a) or FcγRIIIB (CD16b) (relative to the binding exhibited by the wild-type IgG1 Fc Region (**SEQ ID NO:1**). Variant Fc Regions and mutant forms capable of mediating such altered binding are described above. In a specific embodiment, the Fc Region-containing molecules of the present invention comprise an IgG Fc Region that exhibits reduced ADCC effector function. In a preferred embodiment the CH2-CH3 Domain of the first and/or third polypeptide chains of such Fc Region-containing molecules include any 1, 2, or 3, of the substitutions: L234A, L235A, N297Q, and N297G. In another embodiment, the human IgG Fc Region variant contains an N297Q substitution, an N297G substitution, L234A and L235A substitutions or a D265A substitution, as these mutations abolish FcR binding. Alternatively, a CH2-CH3 Domain of an Fc region which inherently exhibits decreased (or substantially no) binding to FcγRIIIA (CD16a) and/or reduced effector function (relative to the binding exhibited by the wild-type IgG1 Fc Region (**SEQ ID NO:1**)) is utilized. In a specific embodiment, the Fc Region-containing molecules of the present invention comprise an IgG2 Fc Region (**SEQ ID NO:2**) or an IgG4 Fc Region (**SEQ ID:NO:4**). When an IgG4 Fc Region in utilized, the instant invention also encompasses the introduction of a stabilizing mutation, such as the hinge region S228P substitution described above (see, *e.g.*, **SEQ ID NO:13**). Since the N297G, N297Q, L234A, L235A and D265A substitutions abolish effector function, in circumstances in which effector function is desired, these substitutions would preferably not be employed.

[00270] In particular, it is preferred for the Fc Regions of the polypeptide chains of the Fc Region-containing molecules of the present invention to exhibit increased serum half-life (relative to the half-life exhibited by the corresponding wild-type Fc). Variant Fc Regions and mutant forms exhibiting extended serum half-life are described above. In a preferred embodiment the CH2-CH3 Domain of the first and/or third polypeptide chains of such Fc Region-containing molecules include any 1, 2, or 3, of the substitutions: M252Y, S254T and T256E. The invention further encompasses Fc Region-containing molecules of the present invention comprising variant Fc Regions comprising:

(A) one or more mutations which alter effector function and/or FcγR; and

(B) one or more mutations which extend serum half-life.

[00271] A preferred IgG1 sequence for the CH2 and CH3 Domains of the Fc Region-containing molecules of the present invention will comprise the substitutions L234A/L235A/M252Y/S254T/T256E (**SEQ ID NO:258**):

APE**AA**GGPSV FLFPPKPKDT L**Y**I**T**R**E**PEVT CVVVDVSHED PEVKFNWYVD
GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE
WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE
ALHNHYTQKS LSLSPG**X**

wherein, X is a lysine (K) or is absent.

[00272] A preferred IgG4 sequence for the CH2 and CH3 Domains of the Fc Region-containing molecules of the present invention will comprise the M252Y/S254T/T256E substitutions (**SEQ ID NO:259**):

APEFLGGPSV FLFPPKPKDT L**Y**I**T**R**E**PEVT CVVVDVSQED PEVQFNWYVD
GVEVHNAKTK PREEQFNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKGLPS
SIEKTISKAK GQPREPQVYT LPPSQEEMTK NQVSLTCLVK GFYPSDIAVE
WESNGQPENN YKTTPPVLDS DGSFFLYSRL TVDKSRWQEG NVFSCSVMHE
ALHNHYTQKS LSLSLG**X**

wherein, X is a lysine (K) or is absent.

[00273] For diabodies and trivalent binding molecules whose first and third polypeptide chains are not identical), it is desirable to reduce or prevent homodimerization from occurring between the CH2-CH3 Domains of two first polypeptide chains or between the CH2-CH3 Domains of two third polypeptide chains. The CH2 and/or CH3 Domains of such polypeptide chains need not be identical in sequence, and advantageously are modified to foster complexing between the two polypeptide chains. For example, an amino acid substitution (preferably a substitution with an amino acid comprising a bulky side group forming a "knob", *e.g.*,

tryptophan) can be introduced into the CH2 or CH3 Domain such that steric interference will prevent interaction with a similarly mutated domain and will obligate the mutated domain to pair with a domain into which a complementary, or accommodating mutation has been engineered, *i.e.*, "the hole" (*e.g.*, a substitution with glycine). Such sets of mutations can be engineered into any pair of polypeptides comprising CH2-CH3 Domains that forms an Fc Region. Methods of protein engineering to favor heterodimerization over homodimerization are well known in the art, in particular with respect to the engineering of immunoglobulin-like molecules, and are encompassed herein (see *e.g.*, Ridgway *et al.* (1996) *"'Knobs-Into-Holes' Engineering Of Antibody CH3 Domains For Heavy Chain Heterodimerization,"* Protein Engr. 9:617-621, Atwell *et al.* (1997) *"Stable Heterodimers From Remodeling The Domain Interface Of A Homodimer Using A Phage Display Library,"* J. Mol. Biol. 270: 26-35, and Xie *et al.* (2005) *"A New Format Of Bispecific Antibody: Highly Efficient Heterodimerization, Expression And Tumor Cell Lysis,"* J. Immunol. Methods 296:95-101; each of which is hereby incorporated herein by reference in its entirety). Preferably the "knob" is engineered into the CH2-CH3 Domains of the first polypeptide chain and the "hole" is engineered into the CH2-CH3 Domains of the third polypeptide chain of diabodies comprising three polypeptide chains. Thus, the "knob" will help in preventing the first polypeptide chain from homodimerizing via its CH2 and/or CH3 Domains. As the third polypeptide chain preferably contains the "hole" substitution it will heterodimerize with the first polypeptide chain as well as homodimerize with itself. This strategy may be utilized for diabodies and trivalent binding molecules comprising three, four or five chains as detailed above, where the "knob" is engineered into the CH2-CH3 Domains of the first polypeptide chain and the "hole" is engineered into the CH2-CH3 Domains the third polypeptide chain.

[00274] A preferred knob is created by modifying an IgG Fc Region to contain the modification T366W. A preferred hole is created by modifying an IgG Fc Region to contain the modification T366S, L368A and Y407V. To aid in purifying the hole-bearing third polypeptide chain homodimer from the final bispecific heterodimeric Fc Region-containing molecule, the protein A binding site of the hole-bearing CH2 and CH3 Domains of the third polypeptide chain is preferably mutated by amino acid substitution at position 435 (H435R). Thus, the hole-bearing third polypeptide chain homodimer will not bind to protein A, whereas the bispecific heterodimer will retain its ability to bind protein A via the protein A binding site on the first polypeptide chain. In an alternative embodiment, the hole-bearing third polypeptide chain may incorporate amino acid substitutions at positions 434 and 435 (N434A/N435K).

[00275] A preferred IgG1 amino acid sequence for the CH2 and CH3 Domains of the first polypeptide chain of an Fc Region-containing molecule of the present invention will have the "**knob-bearing**" sequence (**SEQ ID NO:6**):

APE**AA**GGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD
GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSL**W**CLVK GFYPSDIAVE
WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE
ALHNHYTQKS LSLSPG**X**

wherein, **X** is a lysine (K) or is absent.

[00276] A preferred IgG1 amino acid sequence for the CH2 and CH3 Domains of the second polypeptide chain of an Fc Region-containing molecule of the present invention having two polypeptide chains (or the third polypeptide chain of an Fc Region-containing molecule having three, four, or five polypeptide chains) will have the "**hole-bearing**" sequence (**SEQ ID NO:7**):

APE**AA**GGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD
GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSL**S**C**A**VK GFYPSDIAVE
WESNGQPENN YKTTPPVLDS DGSFFL**V**SKL TVDKSRWQQG NVFSCSVMHE
ALHN**R**YTQKS LSLSPG**X**

wherein, **X** is a lysine (K) or is absent.

[00277] As will be noted, the CH2-CH3 Domains of **SEQ ID NO:6,** and **SEQ ID NO:7** include a substitution at position 234 with alanine and 235 with alanine, and thus form an Fc Region exhibit decreased (or substantially no) binding to FcγRIA (CD64), FcγRIIA (CD32A), FcγRIIB (CD32B), FcγRIIIA (CD16a) or FcγRIIIB (CD16b) (relative to the binding exhibited by the wild-type Fc Region (**SEQ ID NO:1**). The invention also encompasses such CH2-CH3 Domains, which comprise alternative and/or additional substitutions which modify effector function and/or FγR binding activity of the Fc region. The invention also encompasses such CH2-CH3 Domains, which further comprise one or more half-live extending amino acid substitutions. In particular, the invention encompasses such hole-bearing and such knob-bearing CH2-CH3 Domains which further comprise the M252Y/S254T/T256E.

[00278] It is preferred that the first polypeptide chain will have a "knob-bearing" CH2-CH3 sequence, such as that of **SEQ ID NO:6**. However, as will be recognized, a "hole-bearing" CH2-CH3 Domain (*e.g.*, **SEQ ID NO:7**) could be employed in the first polypeptide chain, in which case, a "knob-bearing" CH2-CH3 Domain (*e.g.*, **SEQ ID NO:6**) would be employed in the second polypeptide chain of an Fc Region-containing molecule of the present invention

having two polypeptide chains (or in the third polypeptide chain of an Fc Region-containing molecule having three, four, or five polypeptide chains).

[00279] As detailed above the invention encompasses Fc Region-containing molecules (*e.g.*, antibodies and Fc Region-containing diabodies) having wild type CH2 and CH3 Domains, or having CH2 and CH3 Domains comprising combinations of the substitutions described above. An exemplary amino acid sequence of an IgG1 CH2-CH3 Domain encompassing such variations is (**SEQ ID NO:260**):

APEX_1X_2GGPSV FLFPPKPKDT LX_3IX_4RX_5PEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLX_6CX_7VK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLX$_8$SKL TVDKSRWQQG NVFSCSVMHE ALHX_9X_{10}YTQKS LSLSPGX_{11}

wherein:

(a) X_1 and X_2 are both L (wild type), or are both A (decreased FcγR binding);

(b) X_3, X_4, and X_5 respectively are M, S and T (wild type), or are Y, T and E (extended half-life),

(c) X_6, X_7, and X_8 respectively are: T, L and Y (wild type), or are W, L and Y (knob), or S, A and V (hole);

(d) X_9 and X_{10} respectively are N and H (wild type), or are N and R (no protein A binding), or A and K (no protein A binding); and

(e) X_{11} is K or is absent.

[00280] In other embodiments, the invention encompasses PD-1-binding molecules comprising CH2 and/or CH3 Domains that have been engineered to favor heterodimerization over homodimerization using mutations known in the art, such as those disclosed in PCT Publication No. WO 2007/110205; WO 2011/143545; WO 2012/058768; WO 2013/06867, all of which are incorporated herein by reference in their entirety.

VIII. PD-1 x LAG-3 Bispecific Binding Molecules

[00281] The present invention particularly relates to PD-1 x LAG-3 bispecific binding molecules (*e.g.*, bispecific antibodies, bispecific diabodies, *etc.*) comprising an epitope-binding fragment of an anti-PD-1 antibody, and preferably one of the novel anti-human PD-1 antibodies provided herein, and an epitope-binding fragment of an anti-human LAG-3 antibody, preferably one of the novel anti-human LAG-3 antibodies provded herein. The preferred PD-1 x LAG-3 bispecific binding molecules of the present invention possess epitope-binding fragments of antibodies that enable them to be able to coordinately bind to two different epitopes: an epitope of PD-1 and an epitope of LAG-3, so as to attenuate the inhibitory

activities of such molecules. As used herein, such attenuation refers to a decrease of at least 20%, a decrease of at least 50%, a decrease of at least 80%, or a decrease of at least 90% in detectable PD-1 and/or LAG-3 inhibitory activity, or the complete elimination of detectable PD-1 and/or LAG-3 inhibitory activity. Selection of the epitope-binding fragments (*e.g.*, VL and VH Domains) of the anti-human PD-1 antibody and anti-LAG-3 antibody is coordinated such that the polypeptides chains that make up such PD-1 x LAG-3 bispecific binding molecules assemble to form at least one functional antigen binding site that is specific for the first antigen (*i.e.*, either PD-1 or LAG-3) and at least one functional antigen binding site that is specific for the second antigen (*i.e.*, either PD-1 or LAG-3, depending upon the identity of the first antigen).

[00282] In a particular embodiment, a PD-1 x LAG-3 bispecific binding molecule of the instant invention is a bispecific diabody, which preferably comprises two, three, four, or five polypeptide chains as described herein. In another particular embodiment, a PD-1 x LAG-3 bispecific binding molecule of the instant invention is a bispecific antibody, which preferably comprises two, three, or four polypeptide chains as described herein (also see, *e.g.*, WO 2007/024715; WO2007/110205; WO 2009/080251; WO 2009/080254; WO 2009/089004; WO 2011/069104; WO 2011/117329; WO 2011/131746; WO 2011/133886; WO 2011/143545; WO 2012/023053; WO 2013/060867, all of which descriptions are incorporated herein by reference in their entirety).

A. Anti-Human LAG-3 Antibodies

[00283] Exemplary antibodies that are immunospecific for human LAG-3 are provided below. Additional desired antibodies may be made by isolating antibody-secreting hybridomas elicited using LAG-3 or a peptide fragment thereof, or by screening recombinant antibody libraries for binding to LAG-3 or a peptide fragment thereof. Human LAG-3 (including a 28 amino acid residue signal sequence (shown underlined) and the 497 amino acid residue mature protein) has the amino acid sequence (**SEQ ID NO:38**):

MWEAQFLGLL FLQPLWVAPV KPLQPGAEVP VVWAQEGAPA QLPCSPTIPL
QDLSLLRRAG VTWQHQPDSG PPAAAPGHPL APGPHPAAPS SWGPRPRRYT
VLSVGPGGLR SGRLPLQPRV QLDERGRQRG DFSLWLRPAR RADAGEYRAA
VHLRDRALSC RLRLRLGQAS MTASPPGSLR ASDWVILNCS FSRPDRPASV
HWFRNRGQGR VPVRESPHHH LAESFLFLPQ VSPMDSGPWG CILTYRDGFN
VSIMYNLTVL GLEPPTPLTV YAGAGSRVGL PCRLPAGVGT RSFLTAKWTP
PGGGPDLLVT GDNGDFTLRL EDVSQAQAGT YTCHIHLQEQ QLNATVTLAI

ITVTPKSFGS PGSLGKLLCE VTPVSGQERF VWSSLDTPSQ RSFSGPWLEA
QEAQLLSQPW QCQLYQGERL LGAAVYFTEL SSPGAQRSGR APGALPAGHL
LLFLILGVLS LLLLVTGAFG FHLWRRQWRP RRFSALEQGI HPPQAQSKIE
ELEQEPEPEP EPEPEPEPEP EPEQL

1. LAG-3 mAb A

[00284] The anti-human LAG-3 antibody BMS-986016 (25F7; Medarex/BMS), designated herein as "**LAG-3 mAb A,**" and variants thereof have been described (see, *e.g.*, WO 2014/008218). The amino acid sequence of the Heavy Chain Variable Domain of **LAG-3 mAb A** has the amino acid sequence (**SEQ ID NO:39**) (CDRs are shown underlined):

QVQLQQWGAG LLKPSETLSL TCAVYGGSFS **DYYWN**WIRQP PGKGLEWIG**E**
INHNGNTNSN PSLKSRVTLS LDTSKNQFSL KLRSVTAADT AVYYCAF**GYS**
DYEYNWFDPW GQGTLVTVSS

[00285] The amino acid sequence of the Light Chain Variable Domain of **LAG-3 mAb A** has the amino acid sequence (**SEQ ID NO:40**) (CDRs are shown underlined):

EIVLTQSPAT LSLSPGERAT LSC**RASQSIS SYLA**WYQQKP GQAPRLLIY**D**
ASNRATGIPA RFSGSGSGTD FTLTISSLEP EDFAVYYC**QQ RSNWPLT**FGQ
GTNLEIK

[00286] Additional murine anti-human LAG-3 antibodies possessing unique binding characteristics have recently been identified (see, United States Patent Application No. 62/172,277). Preferred PD-1 x LAG-3 bispecific binding molecules of the present invention comprise the epitope-binding fragments of the anti-human LAG-3 antibody **LAG-3 mAb 1** or **LAG-3 mAb 6**, antibodies, which bind a novel epitope and do not compete with BMS-986016 for LAG-3 binding. Particularly preferred, are PD-1 x LAG-3 bispecific binding molecules of the present invention which possess a humanized VH and/or VL Domains of LAG-3 mAb 1 or LAG-3 mAb 6.

2. LAG-1 mAb 1

[00287] The amino acid sequence of the VH Domain of LAG-3 mAb 1 (**SEQ ID NO:41**) is shown below (CDR_H residues are shown underlined).

QIQLVQSGPE LKKPGETVKI SCKASGYTFR **NYGMN**WVKQA PGKVLKWMG**W**
INTYTGESTY ADDFEGRFAF SLGTSASTAY LQINILKNED TATYFCAR**ES**
LYDYYSMDYW GQGTSVTVSS

CDR_H1 of LAG-3 mAb 1 (**SEQ ID NO:42**): **RNYGMN**

CDR_H2 of LAG-3 mAb 1 (**SEQ ID NO43**): **WINTYTGESTYADDFEG**

CDR$_H$3 of LAG-3 mAb 1 (**SEQ ID NO:44**): ESLYDYYSMDY

[00288] The amino acid sequence of the VL Domain of LAG-3 mAb 1 (**SEQ ID NO:45**) is shown below (CDR$_L$ residues are shown underlined):

DVVVTQTPLT LSVTIGQPAS ISC**KSSQSLL HSDGKTYLN**W LLQRPGQSPE RLIY**LVSELD S**GVPDRFTGS GSGTDFTLKI SRVEAEDLGV YYC**WQGTHFP YT**FGGGTKLE IK

CDR$_L$1 of LAG-3 mAb 1 (**SEQ ID NO:46**): KSSQSLLHSDGKTYLN

CDR$_L$2 of LAG-3 mAb 1 (**SEQ ID NO:47**): LVSELDS

CDR$_L$3 of LAG-3 mAb 1 (**SEQ ID NO:48**): WQGTHFPYT

[00289] Two exemplary humanized VH Domains of LAG-3 mAb 1 designated herein as "**hLAG-3 mAb 1 VH1,**" and "**hLAG-3 mAb 1 VH2**," and four exemplary humanized VL Domains of LAG-3 mAb 1 "**hLAG-3 mAb 1 VL1**," "**hLAG-3 mAb 1 VL2**," "**hLAG-3 mAb 1 VL3**," and "**hLAG-3 mAb 1 VL4**," are provided below. Any of the humanized VL Domains may be paired with any of the humanized VH Domains to generate a LAG-3 binding domain. Accordingly, any antibody comprising one of the humanized VL Domains paired with the humanized VH Domain is referred to generically as "**hLAG-3 mAb 1**," and particular combinations of humanized VH/VL Domains are referred to by reference to the specific VH/VL Domains, for example a humanized antibody comprising hLAG-3 mAb 1 VH1 and hLAG-3 mAb 1 VL2 is specifically referred to as "**hLAG-3 mAb 1(1.2)**."

[00290] The amino acid sequence of the VH Domain of **hLAG-3 mAb 1 VH1** (**SEQ ID NO:49**) is shown below (CDR$_H$ residues are shown underlined):

QVQLVQSGAE VKKPGASVKV SCKASGYTFT **NYGMN**WVRQA PGQGLEWMG**W INTYTGESTY ADDFEG**RFVF SMDTSASTAY LQISSLKAED TAVYYCAR**ES LYDYYSMDY**W GQGTTVTVSS

[00291] The amino acid sequence of the VH Domain of **hLAG-3 mAb 1 VH2** (**SEQ ID NO:50**) is shown below (CDR$_H$ residues are shown underlined):

QVQLVQSGAE VKKPGASVKV SCKASGYTFT **NYGMN**WVRQA PGQGLEWMG**W INTYTGESTY ADDFEG**RFVF SMDTSASTAY LQISSLKAED TAVYFCAR**ES LYDYYSMDY**W GQGTTVTVSS

[00292] The amino acid sequence of the VL Domain of **hLAG-3 mAb 1 VL1** (**SEQ ID NO:51**) is shown below (CDR$_L$ residues are shown underlined):

DIVMTQTPLS LSVTPGQPAS ISC**KSSQSLL HSDGKTYLN**W LLQKPGQSPE

RLIY**LVSELD S**GVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYC**WQGTHFP YT**FGGGTKVE IK

[00293] The amino acid sequence of the VL Domain of **hLAG-3 mAb 1 VL2 (SEQ ID NO:52)** is shown below (CDR_L residues are shown underlined):

DIVMTQTPLS LSVTPGQPAS ISC**KSSQSLL HSDGKTYLN**W LLQRPGQSPE RLIY**LVSELD S**GVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYC**WQGTHFP YT**FGGGTKVE IK

[00294] The amino acid sequence of the VL Domain of **hLAG-3 mAb 1 VL3 (SEQ ID NO:53)** is shown below (CDR_L residues are shown underlined):

DIVMTQTPLS LSVTPGQPAS ISC**KSSQSLL HSDGKTYLN**W LLQKPGQPPE RLIY**LVSELD S**GVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYC**WQGTHFP YT**FGGGTKVE IK

[00295] The amino acid sequence of the VL Domain of **hLAG-3 mAb 1 VL4 (SEQ ID NO:54)** is shown below (CDR_L residues are shown underlined):

DIVMTQTPLS LSVTPGQPAS ISC**KSSQSLL HSDAKTYLN**W LLQKPGQPPE RLIY**LVSELD S**GVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYC**WQGTHFP YT**FGGGTKVE IK

[00296] The CDR_L1 of the VL Domain of hLAG-3 mAb 1 VL4 comprises an glycine to alanine amino acid substitution and has the amino acid sequence: **KSSQSLLHSDAKTYLN (SEQ ID NO:55)**, the substituted alanine is shown underlined). It is contemplated that a similar substitution may be incorporated into any of the LAG-3 mAb 1 CDR_L1 Domains described above.

3. LAG-3 mAb 6

[00297] The amino acid sequence of the VH Domain of LAG-3 mAb 6 (**SEQ ID NO:56**) is shown below (CDR_H residues are shown underlined):

EVLLQQSGPE LVKPGASVKI PCKASGYTFT **DYNMD**WVKQS HGESLEWIG**D INPDNGVTIY NQKFEG**KATL TVDKSSSTAY MELRSLTSED TAVYYCAR**EA DYFYFDY**WGQ GTTLTVSS

CDR_H1 of LAG-3 mAb 6 (**SEQ ID NO:57**): **DYNMD**

CDR_H2 of LAG-3 mAb 6 (**SEQ ID NO:58**): **DINPDNGVTIYNQKFEG**

CDR_H3 of LAG-3 mAb 6 (**SEQ ID NO:59**): **EADYFYFDY**

[00298] The amino acid sequence of the VL Domain of LAG-3 mAb 6 (**SEQ ID NO:60**) is shown below (CDR residues are shown underlined):

DIVMTQSHRF MSTSVGDRVS ITC**KASQDVS SVVA**WYQQKP GQSPKLLIF**S**
ASYRYTGVPD RFTGSGSGTD FTFTISSVQA ADLAVYYCQQ **HYSTPWT**FGG
GTKLEIK

CDR$_L$1 of LAG-3 mAb 6 (**SEQ ID NO:61**): **KASQDVSSVVA**

CDR$_L$2 of LAG-3 mAb 6 (**SEQ ID NO:62**): **SASYRYT**

CDR$_L$3 of LAG-3 mAb 6 (**SEQ ID NO:63**): **HYSTPWT**

[00299] Two exemplary humanized VH Domains of LAG-3 mAb 6 designated herein as "**hLAG-3 mAb 6 VH1,**" and "**hLAG-3 mAb 6 VH2**," and two exemplary humanized VL Domains of LAG-3 mAb 6 "**hLAG-3 mAb 1 VL1**," and "**hLAG-3 mAb 1 VL2**," are provided below. Any of the humanized VL Domains may be paired with any of the humanized VH Domains to generate a LAG-3 binding domain. Accordingly, any antibody comprising one of the humanized VL Domains paired with the humanized VH Domain is referred to generically as "**hLAG-3 mAb 6**," and particular combinations of humanized VH/VL Domains are referred to by reference to the specific VH/VL Domains, for example a humanized antibody comprising hLAG-3 mAb 6 VH1 and hLAG-3 mAb 6 VL2 is specifically referred to as "**hLAG-3 mAb 6(1.2)**."

[00300] The amino acid sequence of the VH Domain of **hLAG-3 mAb 6 VH1 (SEQ ID NO:294)** is shown below (CDR$_H$ residues are shown underlined):

QVQLVQSGAE VKKPGASVKV SCKASGYTFT **DYNMD**WVRQA PGQGLEWMG**D**
INPDNGVTIY NQKFEGRVTM TTDTSTSTAY MELRSLRSDD TAVYYCAR**EA**
DYFYFDYWGQ GTTLTVSS

[00301] An amino acid sequence of the VH Domain of **hLAG-3 mAb 6 VH2 (SEQ ID NO:295)** is shown below (CDR$_H$ residues are shown underlined):

EVQLVESGGG LVKPGGSLRL SCAASGFTFS **DYNMD**WVRQA PGKGLEWVS**D**
INPDNGVTIY NQKFEGRFTI SRDNAKNSLY LQMNSLRAED TAVYYCAR**EA**
DYFYFDYWGQ GTTLTVSS

[00302] The amino acid sequence of the VL Domain of **hLAG-3 mAb 6 VL1 (SEQ ID NO:296)** is shown below (CDR$_L$ residues are shown underlined):

DIQMTQSPSS LSASVGDRVT ITC**RASQDVS SVVA**WYQQKP GKAPKLLIY**S**
ASYRYTGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ **HYSTPWT**FGG
GTKLEIK

[00303] The amino acid sequence of the VL Domain of **hLAG-3 mAb 6 VL2 (SEQ ID NO:297)** is shown below (CDR$_L$ residues are shown underlined):

DIVMTQSPSS LSASVGDRVT ITC**RASQDVS SVVA**WYQQKP GKAPKLLIY**S**
ASYRYTGVPD RFSGSGSGTD FTFTISSLQP EDIAVYYCQQ **HYSTPWT**FGG
GTKLEIK

[00304] The CDR_L1 of the VL Domain of hLAG-3 mAb 6 VL1 and VL2 comprises a lysine to arginine amino acid substitution and has the amino acid sequence: **RASQDVSSVVA** (**SEQ ID NO:298**), the substituted arginine is shown underlined). It is contemplated that a similar substitution may be incorporated into any of the LAG-3 mAb 6 CDR_L1 Domains described above.

B. Exemplary Four Chain Fc Region-Containing Diabodies Having E/K-Coils

[00305] Four exemplary PD-1 X LAG-3 bispecific, four chain Fc Region-containing diabodies comprising E/K-coil Heterodimer-Promoting Domains (designated "**DART A**," "**DART B**," "**DART C**," and "**DART I**") were generated. The structure of these Fc Region-containing diabodies is detailed below. These exemplary PD-1 x LAG-3 diabodies are intended to illustrate, but in no way limit, the scope of the invention.

1. DART A

[00306] DART A is a bispecific, four chain, Fc Region-containing diabody having two binding sites specific for PD-1, two binding sites specific for LAG-3, a variant IgG4 Fc Region engineered for extended half-life, and cysteine-containing E/K-coil Heterodimer-Promoting Domains. The first and third polypeptide chains of DART A comprise, in the N-terminal to C-terminal direction: an N-terminus, a VL Domain of a monoclonal antibody capable of binding to LAG-3 (VL_{LAG-3} hLAG-3 mAb 1 VL4) (**SEQ ID NO:54**); an intervening linker peptide (**Linker 1:** GGGSGGGG (**SEQ ID NO:14**)); a VH Domain of a monoclonal antibody capable of binding to PD-1 (VH_{PD-1} hPD-1 mAb 7 VH1) (**SEQ ID NO:147**); a cysteine-containing intervening linker peptide (**Linker 2:** GGCGGG (**SEQ ID NO:15**)); a cysteine-containing Heterodimer-Promoting (E-coil) Domain (EVAACEK-EVAALEK-EVAALEK-EVAALEK (**SEQ ID NO:23**)); a stabilized IgG4 hinge region (**SEQ ID NO:13**); a variant IgG4 CH2-CH3 Domain comprising substitutions M252Y/S254T/T256E and lacking the C-terminal residue (**SEQ ID NO:259**); and a C-terminus.

[00307] The amino acid sequence of the first and third polypeptide chains of DART A is a variant of **SEQ ID NO:267**:

DIVMTQTPLS LSVTPGQPAS ISCKSSQSLL HSDX$_1$KTYLNW LLQKPGQPPE
RLIYLVSELD SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCWQGTHFP
YTFGGGTKVE IKGGGSGGGG QVQLVQSGAE VKKPGASVKV SCKASGYSFT
SYWMNWVRQA PGQGLEWIGV IHPSDSETWL DQKFKDRVTI TVDKSTSTAY
MELSSLRSED TAVYYCAREH YGTSPFAYWG QGTLVTVSSG GCGGGEVAAC
EKEVAALEKE VAALEKEVAA LEKESKYGPP CPPCPAPEFL GGPSVFLFPP
KPKDTLX$_2$IX$_3$R X$_4$PEVTCVVVD VSQEDPEVQF NWYVDGVEVH NAKTKPREEQ
FNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KGLPSSIEKT ISKAKGQPRE
PQVYTLPPSQ EEMTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTP
PVLDSDGSFF LYSRLTVDKS RWQEGNVFSC SVMHEALHNH YTQKSLSLSL
G

wherein X_1, X_2, X_3 and X_4 are independently selected, and wherein X_1 is A or G; X_2 is Y or M; X_3 is T or S; and X_4 is E or T.

[00308] The amino acid sequences of the first and third polypeptide chains of DART A is **SEQ ID NO:267,** wherein X_1 is A; X_2 is Y; X_3 is T; and X_4 is E.

[00309] The second and fourth polypeptide chains of DART A comprise, in the N-terminal to C-terminal direction: an N-terminus, a VL Domain of a monoclonal antibody capable of binding to PD-1 (VL_{PD-1} hPD-1 mAb 7 VL2) (**SEQ ID NO:153**); an intervening linker peptide (**Linker 1:** GGGSGGGG (**SEQ ID NO:14**)); a VH Domain of a monoclonal antibody capable of binding LAG-3 (VH_{LAG-3} hLAG-3 mAb 1 VH1) (**SEQ ID NO:49**); a cysteine-containing intervening linker peptide (**Linker 2:** GGCGGG (**SEQ ID NO:15**)); a cysteine-containing Heterodimer-Promoting (K-coil) Domain (KVAACKE-KVAALKE-KVAALKE-KVAALKE (**SEQ ID NO:24**); and a C-terminus.

[00310] The amino acid sequence of the second and fourth polypeptide chains of DART A is (**SEQ ID NO:268**):

EIVLTQSPAT LSLSPGERAT LSCRASESVD NYGMSFMNWF QQKPGQPPKL
LIHAASNQGS GVPSRFSGSG SGTDFTLTIS SLEPEDFAVY FCQQSKEVPY
TFGGGTKVEI KGGGSGGGGQ VQLVQSGAEV KKPGASVKVS CKASGYTFTN
YGMNWVRQAP GQGLEWMGWI NTYTGESTYA DDFEGRFVFS MDTSASTAYL
QISSLKAEDT AVYYCARESL YDYYSMDYWG QGTTVTVSSG GCGGGKVAAC
KEKVAALKEK VAALKEKVAA LKE

2. DART B

[00311] DART B is identical to DART A, except that the first and third polypeptide chains of DART B comprise the VL Domain of hLAG-3 mAb 1 VL3 (**SEQ ID NO:53**), which comprises an amino acid substitution in CDR_L1. Thus, the first and third polypeptide chains of DART B comprise, in the N-terminal to C-terminal direction: an N-terminus; a VL Domain

of a monoclonal antibody capable of binding to LAG-3 (VL_{LAG-3} hLAG-3 mAb 1 VL3) (**SEQ ID NO:53**); an intervening linker peptide (**Linker 1:** GGGSGGGG (**SEQ ID NO:14**)); a VH Domain of a monoclonal antibody capable of binding to PD-1 (VH_{PD-1} hPD-1 mAb 7 VH1) (**SEQ ID NO:147**); an intervening linker peptide (**Linker 2:** GGCGGG (**SEQ ID NO:15**)); a cysteine-containing Heterodimer-Promoting (E-coil) Domain (EVAACEK-EVAALEK-EVAALEK-EVAALEK (**SEQ ID NO:23**)); a stabilized IgG4 hinge region (**SEQ ID NO:13**); a variant of IgG4 CH2-CH3 Domain comprising substitutions M252Y/S254T/T256E and lacking the C-terminal residue (**SEQ ID NO:259**); and a C-terminus.

[00312] The amino acid sequence of the first and third polypeptide chains of DART B is **SEQ ID NO:267**, wherein X_1 is G; X_2 is Y; X_3 is T; and X_4 is E.

[00313] The amino acid sequence of the second and fourth polypeptide chains of DART B is **SEQ ID NO:268**.

3. DART C

[00314] DART C is identical to DART B, except that the first and third polypeptide chains of DART B comprise a wild type IgG4 CH2-CH3 Domain lacking the C-terminal residue (**SEQ ID NO:4**). Thus, the first and third polypeptide chains of DART C comprise, in the N-terminal to C-terminal direction: an N-terminus, a VL Domain of a monoclonal antibody capable of binding to LAG-3 (VL_{LAG-3} hLAG-3 mAb 1 VL3) (**SEQ ID NO:53**); an intervening linker peptide (**Linker 1:** GGGSGGGG (**SEQ ID NO:14**)); a VH Domain of a monoclonal antibody capable of binding to PD-1 (VH_{PD-1} hPD-1 mAb 7 VH1) (**SEQ ID NO:147**); an intervening linker peptide (**Linker 2:** GGCGGG (**SEQ ID NO:15**)); a cysteine-containing Heterodimer-Promoting (E-coil) Domain (EVAACEK-EVAALEK-EVAALEK-EVAALEK (**SEQ ID NO:23**)); a stabilized IgG4 hinge region (**SEQ ID NO:13**); an IgG4 CH2-CH3 Domain lacking the C-terminal residue (**SEQ ID NO:4**); and a C-terminus.

[00315] The amino acid sequence of the first and third polypeptide chains of DART C is **SEQ ID NO:267**, wherein X_1 is G; X_2 is M; X_3 is S; and X_4 is T.

[00316] The amino acid sequence of the second and fourth polypeptide chains of DART C is **SEQ ID NO:268**.

4. DART I

[00317] DART I is a bispecific, four chain, Fc Region-containing diabody having two binding sites specific for PD-1, two binding sites specific for LAG-3, a variant IgG4 Fc Region engineered for extended half-life, and cysteine-containing E/K-coil Heterodimer-Promoting Domains. The first and third polypeptide chains of DART I comprise, in the N-terminal to C-terminal direction: an N-terminus, a VL Domain of a monoclonal antibody capable of binding to LAG-3 (VL_{LAG-3} hLAG-3 mAb 6 VL1) (**SEQ ID NO:296**); an intervening linker peptide (**Linker 1:** GGGSGGGG (**SEQ ID NO:14**)); a VH Domain of a monoclonal antibody capable of binding to PD-1 (VH_{PD-1} hPD-1 mAb 7 VH1) (**SEQ ID NO:147**); a cysteine-containing intervening linker peptide (**Linker 2:** GGCGGG (**SEQ ID NO:15**)); a cysteine-containing Heterodimer-Promoting (E-coil) Domain (EVAACEK-EVAALEK-EVAALEK-EVAALEK (**SEQ ID NO:23**)); a stabilized IgG4 hinge region (**SEQ ID NO:13**); a variant IgG4 CH2-CH3 Domain comprising substitutions M252Y/S254T/T256E and lacking the C-terminal residue (**SEQ ID NO:259**); and a C-terminus.

[00318] The amino acid sequence of the first and third polypeptide chains of DART I is (**SEQ ID NO:290**):

DIQMTQSPSS LSASVGDRVT ITCRASQDVS SVVAWYQQKP GKAPKLLIYS
ASYRYTGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ HYSTPWTFGG
GTKLEIKGGG SGGGGQVQLV QSGAEVKKPG ASVKVSCKAS GYSFTSYWMN
WVRQAPGQGL EWIGVIHPSD SETWLDQKFK DRVTITVDKS TSTAYMELSS
LRSEDTAVYY CAREHYGTSP FAYWGQGTLV TVSSGGCGGG EVAACEKEVA
ALEKEVAALE KEVAALEKES KYGPPCPPCP APEFLGGPSV FLFPPKPKDT
LYITREPEVT CVVVDVSQED PEVQFNWYVD GVEVHNAKTK PREEQFNSTY
RVVSVLTVLH QDWLNGKEYK CKVSNKGLPS SIEKTISKAK GQPREPQVYT
LPPSQEEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS
DGSFFLYSRL TVDKSRWQEG NVFSCSVMHE ALHNHYTQKS LSLSLG

[00319] The second and fourth polypeptide chains of DART I comprise, in the N-terminal to C-terminal direction: an N-terminus, a VL Domain of a monoclonal antibody capable of binding to PD-1 (VL_{PD-1} hPD-1 mAb 7 VL2) (**SEQ ID NO:153**); an intervening linker peptide (**Linker 1:** GGGSGGGG (**SEQ ID NO:14**)); a VH Domain of a monoclonal antibody capable of binding LAG-3 (VH_{LAG-3} hLAG-3 mAb 6 VH1) (**SEQ ID NO:294**); a cysteine-containing intervening linker peptide (**Linker 2:** GGCGGG (**SEQ ID NO:15**)); a cysteine-containing Heterodimer-Promoting (K-coil) Domain (KVAACKE-KVAALKE-KVAALKE-KVAALKE (**SEQ ID NO:24**); and a C-terminus.

[00320] The amino acid sequence of the second and fourth polypeptide chains of DART I is (**SEQ ID NO:291**):

EIVLTQSPAT LSLSPGERAT LSCRASESVD NYGMSFMNWF QQKPGQPPKL
LIHAASNQGS GVPSRFSGSG SGTDFTLTIS SLEPEDFAVY FCQQSKEVPY
TFGGGTKVEI KGGGSGGGGQ VQLVQSGAEV KKPGASVKVS CKASGYTFTD
YNMDWVRQAP GQGLEWMGDI NPDNGVTIYN QKFEGRVTMT TDTSTSTAYM
ELRSLRSDDT AVYYCAREAD YFYFDYWGQG TTLTVSSGGC GGGKVAACKE
KVAALKEKVA ALKEKVAALK E

C. Exemplary Four Chain Fc Region-Containing Diabodies Having CL/CH1 Domains

[00321] Four exemplary PD-1 X LAG-3 bispecific, four chain Fc Region-containing diabodies comprising CL/CH1 Domains designated "**DART D**," "**DART E**," "**DART J**"and "**DART 1**" were generated. The structure of these Fc Region-containing diabodies is detailed below. These exemplary PD-1 x LAG-3 diabodies are intended to illustrate, but in no way limit, the scope of the invention.

1. DART D

[00322] DART D is a bispecific, four chain, Fc Region-containing diabody having two binding sites specific for PD-1, two binding sites specific for LAG-3, CL/CH1 Domains, and a variant IgG4 Fc Region engineered for extended half-life. The first and third polypeptide chains of DART D comprise, in the N-terminal to C-terminal direction: an N-terminus; a VL Domain of a monoclonal antibody capable of binding to PD-1 (VL_{PD-1} hPD-1 mAb 7 VL2) (**SEQ ID NO:153**); an intervening linker peptide (**Linker 1:** GGGSGGGG (**SEQ ID NO:14**)); a VH Domain of a monoclonal antibody capable of binding to LAG-3 (VH_{LAG-3} hLAG-3 mAb 1 VH1) (**SEQ ID NO:49**); an intervening linker peptide (**Linker 2:** LGGGSG (**SEQ ID NO:261**)); an IgG4 CH1 Domain (**SEQ ID NO:254**); a stabilized IgG4 hinge region (**SEQ ID NO: 13**); a variant of an IgG4 CH2-CH3 Domain comprising substitutions M252Y/S254T/T256E and lacking the C-terminal residue (**SEQ ID NO:259**); and a C-terminus.

[00323] The amino acid sequence of the first and third polypeptide chains of DART D is (**SEQ ID NO:269**):

EIVLTQSPAT LSLSPGERAT LSCRASESVD NYGMSFMNWF QQKPGQPPKL
LIHAASNQGS GVPSRFSGSG SGTDFTLTIS SLEPEDFAVY FCQQSKEVPY
TFGGGTKVEI KGGGSGGGGQ VQLVQSGAEV KKPGASVKVS CKASGYTFTN

YGMNWVRQAP GQGLEWMGWI NTYTGESTYA DDFEGRFVFS MDTSASTAYL
QISSLKAEDT AVYYCARESL YDYYSMDYWG QGTTVTVSSL GGGSGASTKG
PSVFPLAPCS RSTSESTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA
VLQSSGLYSL SSVVTVPSSS LGTKTYTCNV DHKPSNTKVD KRVESKYGPP
CPPCPAPEFL GGPSVFLFPP KPKDTLYITR EPEVTCVVVD VSQEDPEVQF
NWYVDGVEVH NAKTKPREEQ FNSTYRVVSV LTVLHQDWLN GKEYKCKVSN
KGLPSSIEKT ISKAKGQPRE PQVYTLPPSQ EEMTKNQVSL TCLVKGFYPS
DIAVEWESNG QPENNYKTTP PVLDSDGSFF LYSRLTVDKS RWQEGNVFSC
SVMHEALHNH YTQKSLSLSL G

[00324] The second and fourth polypeptide chains of DART D comprise, in the N-terminal to C-terminal direction: an N-terminus; a VL Domain of a monoclonal antibody capable of binding to LAG-3 (VL_{LAG-3} hLAG-3 mAb 1 VL4) (**SEQ ID NO:54**); an intervening linker peptide (**Linker 1**: GGGSGGGG (**SEQ ID NO:14**)); a VH Domain of a monoclonal antibody capable of binding PD-1 (VH_{PD-1} hPD-1 mAb 7 VH1) (**SEQ ID NO:147**); an intervening linker peptide (**Linker 2**: LGGGSG (**SEQ ID NO:261**)); a Kappa CL Domain (**SEQ ID NO:8**); and a C-terminus.

[00325] The amino acid sequence of the second and fourth polypeptide chains of DART D is (**SEQ ID NO:270**):

DIVMTQTPLS LSVTPGQPAS ISCKSSQSLL HSDAKTYLNW LLQKPGQPPE
RLIYLVSELD SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCWQGTHFP
YTFGGGTKVE IKGGGSGGGG QVQLVQSGAE VKKPGASVKV SCKASGYSFT
SYWMNWVRQA PGQGLEWIGV IHPSDSETWL DQKFKDRVTI TVDKSTSTAY
MELSSLRSED TAVYYCAREH YGTSPFAYWG QGTLVTVSSL GGGSGRTVAA
PSVFIFPPSD EQLKSGTASV VCLLNNFYPR EAKVQWKVDN ALQSGNSQES
VTEQDSKDST YSLSSTLTLS KADYEKHKVY ACEVTHQGLS SPVTKSFNRG
EC

2. DART E

[00326] DART E is another bispecific, four chain, Fc Region-containing diabody having two binding sites specific for PD-1, two binding sites specific for LAG-3, CL/CH1 Domains, and a variant IgG4 Fc Region engineered for extended half-life. The position of the PD-1 and LAG-3 binding sites of DART E is reversed as compared to DART D.

[00327] The first and third polypeptide chains of DART E comprise, in the N-terminal to C-terminal direction: an N-terminus, a VL Domain of a monoclonal antibody capable of binding to LAG-3 (VL_{LAG-3} hLAG-3 mAb 1 VL4) (**SEQ ID NO:54**); an intervening linker peptide (**Linker 1:** GGGSGGGG (**SEQ ID NO:14**)); a VH Domain of a monoclonal antibody capable

of binding PD-1 (VH_{PD-1} hPD-1 mAb 7 VH1) (**SEQ ID NO:147**); an intervening linker peptide (**Linker 2:** LGGGSG (**SEQ ID NO:261**)); an IgG4 CH1 Domain (**SEQ ID NO:254**); a stabilized IgG4 hinge region (**SEQ ID NO: 13**); a variant of an IgG4 CH2-CH3 Domain comprising substitutions M252Y/S254T/T256E and lacking the C-terminal residue (**SEQ ID NO:259**); and a C-terminus.

[00328] The amino acid sequence of the first and third polypeptide chains of DART E is (**SEQ ID NO:271**):

DIVMTQTPLS LSVTPGQPAS ISCKSSQSLL HSDAKTYLNW LLQKPGQPPE
RLIYLVSELD SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCWQGTHFP
YTFGGGTKVE IKGGGSGGGG QVQLVQSGAE VKKPGASVKV SCKASGYSFT
SYWMNWVRQA PGQGLEWIGV IHPSDSETWL DQKFKDRVTI TVDKSTSTAY
MELSSLRSED TAVYYCAREH YGTSPFAYWG QGTLVTVSSL GGGSGASTKG
PSVFPLAPCS RSTSESTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA
VLQSSGLYSL SSVVTVPSSS LGTKTYTCNV DHKPSNTKVD KRVESKYGPP
CPPCPAPEFL GGPSVFLFPP KPKDTLYITR EPEVTCVVVD VSQEDPEVQF
NWYVDGVEVH NAKTKPREEQ FNSTYRVVSV LTVLHQDWLN GKEYKCKVSN
KGLPSSIEKT ISKAKGQPRE PQVYTLPPSQ EEMTKNQVSL TCLVKGFYPS
DIAVEWESNG QPENNYKTTP PVLDSDGSFF LYSRLTVDKS RWQEGNVFSC
SVMHEALHNH YTQKSLSLSL G

[00329] The second and fourth polypeptide chains of DART E comprise, in the N-terminal to C-terminal direction: an N-terminus; a VL Domain of a monoclonal antibody capable of binding to PD-1 (VL_{PD-1} hPD-1 mAb 7 VL2) (**SEQ ID NO:153**); an intervening linker peptide (**Linker 1:** GGGSGGGG (**SEQ ID NO:14**)); a VH Domain of a monoclonal antibody capable of binding to LAG-3 (VH_{LAG-3} hLAG-3 mAb 1 VH1) (**SEQ ID NO:49**); an intervening linker peptide (**Linker 2:** LGGGSG (**SEQ ID NO:261**)); a Kappa CL Domain (**SEQ ID NO:8**), and a C-terminus.

[00330] The amino acid sequence of the second and fourth polypeptide chains of DART E is (**SEQ ID NO:272**):

EIVLTQSPAT LSLSPGERAT LSCRASESVD NYGMSFMNWF QQKPGQPPKL
LIHAASNQGS GVPSRFSGSG SGTDFTLTIS SLEPEDFAVY FCQQSKEVPY
TFGGGTKVEI KGGGSGGGGQ VQLVQSGAEV KKPGASVKVS CKASGYTFTN
YGMNWVRQAP GQGLEWMGWI NTYTGESTYA DDFEGRFVFS MDTSASTAYL
QISSLKAEDT AVYYCARESL YDYYSMDYWG QGTTVTVSSL GGGSGRTVAA
PSVFIFPPSD EQLKSGTASV VCLLNNFYPR EAKVQWKVDN ALQSGNSQES
VTEQDSKDST YSLSSTLTLS KADYEKHKVY ACEVTHQGLS SPVTKSFNRG
EC

3. DART J

[00331] DART J is a bispecific, four chain, Fc Region-containing diabody having two binding sites specific for PD-1, two binding sites specific for LAG-3, CL/CH1 Domains, and a variant IgG4 Fc Region engineered for extended half-life. The first and third polypeptide chains of DART J comprise, in the N-terminal to C-terminal direction: an N-terminus; a VL Domain of a monoclonal antibody capable of binding to LAG-3 (VL_{LAG-3} hLAG-3 mAb 6 VL1) (**SEQ ID NO:296**); an intervening linker peptide (**Linker 1**: GGGSGGGG (**SEQ ID NO:14**)); a VH Domain of a monoclonal antibody capable of binding PD-1 (VH_{PD-1} hPD-1 mAb 7 VH1) (**SEQ ID NO:147**); an intervening linker peptide (**Linker 2:** LGGGSG (**SEQ ID NO:261**)); an IgG4 CH1 Domain (**SEQ ID NO:254**); a stabilized IgG4 hinge region (**SEQ ID NO: 13**); a variant of an IgG4 CH2-CH3 Domain comprising substitutions M252Y/S254T/T256E and lacking the C-terminal residue (**SEQ ID NO:259**); and a C-terminus.

[00332] The amino acid sequence of the first and third polypeptide chains of DART J is (**SEQ ID NO:292**):

DIQMTQSPSS LSASVGDRVT ITCRASQDVS SVVAWYQQKP GKAPKLLIYS
ASYRYTGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ HYSTPWTFGG
GTKLEIKGGG SGGGGQVQLV QSGAEVKKPG ASVKVSCKAS GYSFTSYWMN
WVRQAPGQGL EWIGVIHPSD SETWLDQKFK DRVTITVDKS TSTAYMELSS
LRSEDTAVYY CAREHYGTSP FAYWGQGTLV TVSSLGGGSG ASTKGPSVFP
LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS
GLYSLSSVVT VPSSSLGTKT YTCNVDHKPS NTKVDKRVES KYGPPCPPCP
APEFLGGPSV FLFPPKPKDT LYITREPEVT CVVVDVSQED PEVQFNWYVD
GVEVHNAKTK PREEQFNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKGLPS
SIEKTISKAK GQPREPQVYT LPPSQEEMTK NQVSLTCLVK GFYPSDIAVE
WESNGQPENN YKTTPPVLDS DGSFFLYSRL TVDKSRWQEG NVFSCSVMHE
ALHNHYTQKS LSLSLG

[00333] The second and fourth polypeptide chains of DART J comprise, in the N-terminal to C-terminal direction: an N-terminus; a VL Domain of a monoclonal antibody capable of binding to PD-1 (VL_{PD-1} hPD-1 mAb 7 VL2) (**SEQ ID NO:153**); an intervening linker peptide (**Linker 1:** GGGSGGGG (**SEQ ID NO:14**)); a VH Domain of a monoclonal antibody capable of binding to LAG-3 (VH_{LAG-3} hLAG-3 mAb 6 VH1) (**SEQ ID NO:294**); an intervening linker peptide (**Linker 2**: LGGGSG (**SEQ ID NO:261**)); a Kappa CL Domain (**SEQ ID NO:8**); and a C-terminus.

[00334] The amino acid sequence of the second and fourth polypeptide chains of DART J is (**SEQ ID NO:293**):

EIVLTQSPAT LSLSPGERAT LSCRASESVD NYGMSFMNWF QQKPGQPPKL
LIHAASNQGS GVPSRFSGSG SGTDFTLTIS SLEPEDFAVY FCQQSKEVPY
TFGGGTKVEI KGGGSGGGGQ VQLVQSGAEV KKPGASVKVS CKASGYTFTD
YNMDWVRQAP GQGLEWMGDI NPDNGVTIYN QKFEGRVTMT TDTSTSTAYM
ELRSLRSDDT AVYYCAREAD YFYFDYWGQG TTLTVSSLGG GSGRTVAAPS
VFIFPPSDEQ LKSGTASVVC LLNNFYPREA KVQWKVDNAL QSGNSQESVT
EQDSKDSTYS LSSTLTLSKA DYEKHKVYAC EVTHQGLSSP VTKSFNRGEC

4. DART 1

[00335] DART 1 is a bispecific, four chain, Fc Region-containing diabody having two binding sites specific for PD-1, two binding sites specific for LAG-3, CL/CH1 Domains, and a variant IgG1 Fc Region engineered for reduced FcγR binding. The first and third polypeptide chains of DART 1 comprise, in the N-terminal to C-terminal direction: an N-terminus; a VL Domain of a monoclonal antibody capable of binding to PD-1 (VL_{PD-1} PD-1 mAb A VL) (**SEQ ID NO:65**); an intervening linker peptide (**Linker 1:** GGGSGGGG (**SEQ ID NO:14**)); a VH Domain of a monoclonal antibody capable of binding to LAG-3 (VH_{LAG-3} LAG-3 mAb A VH1) (**SEQ ID NO:39**); an intervening linker peptide (**Linker 2:** LGGGSG (**SEQ ID NO:261**)); an IgG1 CH1 Domain (**SEQ ID NO:10**); an IgG1 hinge region (**SEQ ID NO: 32**); a variant of an IgG1 CH2-CH3 Domain comprising substitutions L234A/L235A and lacking the C-terminal residue (**SEQ ID NO:5**); and a C-terminus.

[00336] The amino acid sequence of the first and third polypeptide chains of DART 1 is (**SEQ ID NO:284**):

EIVLTQSPAT LSLSPGERAT LSCRASQSIS SYLAWYQQKP GQAPRLLIYD
ASNRATGIPA RFSGSGSGTD FTLTISSLEP EDFAVYYCQQ RSNWPLTFGQ
GTNLEIKGGG SGGGGQVQLV ESGGGVVQPG RSLRLDCKAS GITFSNSGMH
WVRQAPGKGL EWVAVIWYDG SKRYYADSVK GRFTISRDNS KNTLFLQMNS
LRAEDTAVYY CATNDDYWGQ GTLVTVSSLG GGSGASTKGP SVFPLAPSSK
STSGGTAALG CLVKDYFPEP VTVSWNSGAL TSGVHTFPAV LQSSGLYSLS
SVVTVPSSSL GTQTYICNVN HKPSNTKVDK RVEPKSCDKT HTCPPCPAPE
AAGGPSVFLF PPKPKDTLYI TREPEVTCVV VDVSHEDPEV KFNWYVDGVE
VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV SNKALPAPIE
KTISKAKGQP REPQVYTLPP SREEMTKNQV SLTCLVKGFY PSDIAVEWES
NGQPENNYKT TPPVLDSDGS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH
NHYTQKSLSL SPG

[00337] The second and fourth polypeptide chains of DART 1 comprise, in the N-terminal to C-terminal direction: an N-terminus; a VL Domain of a monoclonal antibody capable of binding to LAG-3 (VL_{LAG-3} LAG-3 mAb A VL) (**SEQ ID NO:40**); an intervening linker peptide (**Linker 1**: GGGSGGGG (**SEQ ID NO:14**)); a VH Domain of a monoclonal antibody capable of binding PD-1 (VH_{PD-1} PD-1 mAb A VH) (**SEQ ID NO:64**); an intervening linker peptide (**Linker 2**: LGGGSG (**SEQ ID NO:261**)); a Kappa CL Domain (**SEQ ID NO:8**); and a C-terminus.

[00338] The amino acid sequence of the second and fourth polypeptide chains of DART 1 is (**SEQ ID NO:285**):

EIVLTQSPAT LSLSPGERAT LSCRASQSVS SYLAWYQQKP GQAPRLLIYD
ASNRATGIPA RFSGSGSGTD FTLTISSLEP EDFAVYYCQQ SSNWPRTFGQ
GTKVEIKGGG SGGGGQVQLQ QWGAGLLKPS ETLSLTCAVY GGSFSDYYWN
WIRQPPGKGL EWIGEINHNG NTNSNPSLKS RVTLSLDTSK NQFSLKLRSV
TAADTAVYYC AFGYSDYEYN WFDPWGQGTL VTVSSLGGGS GRTVAAPSVF
IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ
DSKDSTYSLS STLTLSKADY EKHKVYACEV THQGLSSPVT KSFNRGEC

D. Exemplary Five Chain Fc Region-Containing Diabodies

[00339] Two exemplary PD-1 X LAG-3 bispecific, five chain Fc Region-containing diabodies comprising CL/CH1 Domains and E/K-coil Heterodimer-Promoting Domains designated "**DART F**," and "**DART G**" were generated. The structure of these Fc Region-containing diabodies is detailed below. These exemplary PD-1 x LAG-3 diabodies are intended to illustrate, but in no way limit, the scope of the invention.

1. DART F

[00340] DART F is a bispecific, five chain, Fc Region-containing diabody having three binding sites specific for PD-1, one binding site specific for LAG-3, CL/CH1 Domains, a variant knob/hole-bearing IgG1 Fc Region engineered for reduced FcγR binding and extended half-life, and E/K-coil Heterodimer-Promoting Domains. The first polypeptide chain of DART F comprises, in the N-terminal to C-terminal direction: an N-terminus; a VH Domain of a monoclonal antibody capable of binding PD-1 (VH_{PD-1} hPD-1 mAb 7 VH1) (**SEQ ID NO:147**); an IgG1 CH1 Domain (**SEQ ID NO:10**); an IgG1 hinge region (**SEQ ID NO:32**); a hole-bearing IgG1 CH2-CH3 Domain comprising substitutions L234A/L235A/M252Y/S254T/T256E/N434A/H435K and lacking the C-terminal residue

(**SEQ ID NO:260,** wherein X_1 is A, X_2 is A; X_3 is Y, X_4 is T, X_5 is E, X_6 is S, X_7 is A, X_8 is V, X_9 is A, X_{10} is K, and X_{11} is absent); and a C-terminus.

[00341] The amino acid sequence of the first polypeptide chain of DART F is (**SEQ ID NO:273**):

QVQLVQSGAE VKKPGASVKV SCKASGYSFT SYWMNWVRQA PGQGLEWIGV
IHPSDSETWL DQKFKDRVTI TVDKSTSTAY MELSSLRSED TAVYYCAREH
YGTSPFAYWG QGTLVTVSSA STKGPSVFPL APSSKSTSGG TAALGCLVKD
YFPEPVTVSW NSGALTSGVH TFPAVLQSSG LYSLSSVVTV PSSSLGTQTY
ICNVNHKPSN TKVDKRVEPK SCDKTHTCPP CPAPEAAGGP SVFLFPPKPK
DTLYITREPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS
TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV
YTLPPSREEM TKNQVSLSCA VKGFYPSDIA VEWESNGQPE NNYKTTPPVL
DSDGSFFLVS KLTVDKSRWQ QGNVFSCSVM HEALHAKYTQ KSLSLSPG

[00342] The second and fifth polypeptide chains of DART F comprise, in the N-terminal to C-terminal direction: an N-terminus; a VL Domain of a monoclonal antibody capable of binding to PD-1 (VL_{PD-1} hPD-1 mAb 7 VL2) (**SEQ ID NO:153**), a Kappa CL Domain (**SEQ ID NO:8**), and a C-terminus.

[00343] The amino acid sequence of the second and fifth polypeptide chain of DART F is (**SEQ ID NO:274**):

EIVLTQSPAT LSLSPGERAT LSCRASESVD NYGMSFMNWF QQKPGQPPKL
LIHAASNQGS GVPSRFSGSG SGTDFTLTIS SLEPEDFAVY FCQQSKEVPY
TFGGGTKVEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV
QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV
THQGLSSPVT KSFNRGEC

[00344] The third polypeptide chain of DART F comprises, in the N-terminal to C-terminal direction: an N-terminus; a VH Domain of a monoclonal antibody capable of binding PD-1 (VH_{PD-1} hPD-1 mAb 7 VH1) (**SEQ ID NO:147**); an IgG1 CH1 Domain (**SEQ ID NO:10**); an IgG1 hinge region (**SEQ ID NO:32**); a knob-bearing IgG1 CH2-CH3 Domain comprising substitutions L234A/L235A/M252Y/S254T/T256E and lacking the C-terminal residue (**SEQ ID NO:260,** wherein X_1 is A, X_2 is A; X_3 is Y, X_4 is T, X_5 is E, X_6 is W, X_7 is L, X_8 is Y, X_9 is N, X_{10} is H, and X_{11} is absent); an intervening linker peptide (GGGSGGGSGGG (**SEQ ID NO:262**)); a VL Domain of a monoclonal antibody capable of binding to LAG-3 (VL_{LAG-3} hLAG-3 mAb 1 VL4) (**SEQ ID NO:54**); an intervening linker peptide (**Linker 1:** GGGSGGGG (**SEQ ID NO:14**)); a VH Domain of a monoclonal antibody capable of binding PD-1 (VH_{PD-1} hPD-1 mAb 7 VH1) (**SEQ ID NO:147**); a cysteine-containing intervening linker peptide

(**Linker 2:** GGCGGG (**SEQ ID NO:15**)); a Heterodimer-Promoting (E-coil) Domain (EVAALEK-EVAALEK-EVAALEK-EVAALEK (**SEQ ID NO:21**)); and a C-terminus.

[00345] The amino acid sequence of the third polypeptide chain of DART F is (**SEQ ID NO:275**):

QVQLVQSGAE VKKPGASVKV SCKASGYSFT SYWMNWVRQA PGQGLEWIGV
IHPSDSETWL DQKFKDRVTI TVDKSTSTAY MELSSLRSED TAVYYCAREH
YGTSPFAYWG QGTLVTVSSA STKGPSVFPL APSSKSTSGG TAALGCLVKD
YFPEPVTVSW NSGALTSGVH TFPAVLQSSG LYSLSSVVTV PSSSLGTQTY
ICNVNHKPSN TKVDKRVEPK SCDKTHTCPP CPAPEAAGGP SVFLFPPKPK
DTLYITREPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS
TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV
YTLPPSREEM TKNQVSLWCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL
DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPGGG
GSGGGSGGGD IVMTQTPLSL SVTPGQPASI SCKSSQSLLH SDAKTYLNWL
LQKPGQPPER LIYLVSELDS GVPDRFSGSG SGTDFTLKIS RVEAEDVGVY
YCWQGTHFPY TFGGGTKVEI KGGGSGGGGQ VQLVQSGAEV KKPGASVKVS
CKASGYSFTS YWMNWVRQAP GQGLEWIGVI HPSDSETWLD QKFKDRVTIT
VDKSTSTAYM ELSSLRSEDT AVYYCAREHY GTSPFAYWGQ GTLVTVSSGG
CGGGEVAALE KEVAALEKEV AALEKEVAAL EK

[00346] The fourth polypeptide chain of DART F comprises, in the N-terminal to C-terminal direction: an N-terminus; a VL Domain of a monoclonal antibody capable of binding to PD-1 (VL_{PD-1} hPD-1 mAb 7 VL2) (**SEQ ID NO:153**); an intervening linker peptide (**Linker 1:** GGGSGGGG (**SEQ ID NO:14**)); a VH Domain of a monoclonal antibody capable of binding to LAG-3 (VH_{LAG-3} hLAG-3 mAb 1 VH1) (**SEQ ID NO:49**); a cysteine-containing intervening linker peptide (**Linker 2:** GGCGGG (**SEQ ID NO:15**)); a Heterodimer-Promoting (K-coil) Domain (KVAALKE-KVAALKE-KVAALKE-KVAALKE (**SEQ ID NO:22**)); and a C-terminus.

[00347] The amino acid sequence of the fourth polypeptide chains of DART F is (**SEQ ID NO:276**):

EIVLTQSPAT LSLSPGERAT LSCRASESVD NYGMSFMNWF QQKPGQPPKL
LIHAASNQGS GVPSRFSGSG SGTDFTLTIS SLEPEDFAVY FCQQSKEVPY
TFGGGTKVEI KGGGSGGGGQ VQLVQSGAEV KKPGASVKVS CKASGYTFTN
YGMNWVRQAP GQGLEWMGWI NTYTGESTYA DDFEGRFVFS MDTSASTAYL
QISSLKAEDT AVYYCARESL YDYYSMDYWG QGTTVTVSSG GCGGGKVAAL
KEKVAALKEK VAALKEKVAA LKE

2. DART G

[00348] DART G is a bispecific, five chain, Fc Region-containing diabody having two binding sites specific for PD-1, two binding sites specific for LAG-3, CL/CH1 Domains, a variant knob/hole-bearing IgG1 Fc Region engineered for reduced FcγR binding and extended half-life, and E/K-coil Heterodimer-Promoting Domains. The first polypeptide chain of DART G comprises, in the N-terminal to C-terminal direction: an N-terminus; a VH Domain of a monoclonal antibody capable of binding to LAG-3 (VH_{LAG-3} hLAG-3 mAb 1 VH1) (**SEQ ID NO:49**); an IgG1 CH1 Domain (**SEQ ID NO:10**); an IgG1 hinge region (**SEQ ID NO:32**); a hole-bearing IgG1 CH2-CH3 Domain comprising substitutions L234A/L235A/M252Y/S254T/T256E/N434A/H435K and lacking the C-terminal residue (**SEQ ID NO:260,** wherein X_1 is A, X_2 is A; X_3 is Y, X_4 is T, X_5 is E, X_6 is S, X_7 is A, X_8 is V, X_9 is A, X_{10} is K, and X_{11} is absent); and a C-terminus.

[00349] The amino acid sequence of the first polypeptide chain of DART G is (**SEQ ID NO:277**):

QVQLVQSGAE VKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW
INTYTGESTY ADDFEGRFVF SMDTSASTAY LQISSLKAED TAVYYCARES
LYDYYSMDYW GQGTTVTVSS ASTKGPSVFP LAPSSKSTSG GTAALGCLVK
DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT
YICNVNHKPS NTKVDKRVEP KSCDKTHTCP PCPAPEAAGG PSVFLFPPKP
KDTLYITREP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN
STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ
VYTLPPSREE MTKNQVSLSC AVKGFYPSDI AVEWESNGQP ENNYKTTPPV
LDSDGSFFLV SKLTVDKSRW QQGNVFSCSV MHEALHAKYT QKSLSLSPG

[00350] The second and fifth polypeptide chains of DART G comprise, in the N-terminal to C-terminal direction: an N-terminus; a VL Domain of a monoclonal antibody capable of binding to LAG-3 (VL_{LAG-3} hLAG-3 mAb 1 VL4) (**SEQ ID NO:54**), a Kappa CL Domain (**SEQ ID NO:8**), and a C-terminus.

[00351] The amino acid sequence of the second and fifth polypeptide chain of DART G is (**SEQ ID NO:278**):

DIVMTQTPLS LSVTPGQPAS ISCKSSQSLL HSDAKTYLNW LLQKPGQPPE
RLIYLVSELD SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCWQGTHFP
YTFGGGTKVE IKRTVAAPSV FIFPPSDEQL KSGTASVVCL LNNFYPREAK
VQWKVDNALQ SGNSQESVTE QDSKDSTYSL SSTLTLSKAD YEKHKVYACE
VTHQGLSSPV TKSFNRGEC

[00352] The third polypeptide chain of DART G comprises, in the N-terminal to C-terminal direction: an N-terminus; a VH Domain of a monoclonal antibody capable of binding to LAG-3 (VH_{LAG-3} hLAG-3 mAb 1 VH1) (**SEQ ID NO:49**); an IgG1 CH1 Domain (**SEQ ID NO:10**); an IgG1 hinge region (**SEQ ID NO:32**); a knob-bearing IgG1 CH2-CH3 Domain comprising substitutions L234A/L235A/M252Y/S254T/T256E and lacking the C-terminal residue (**SEQ ID NO:260,** wherein X_1 is A, X_2 is A; X_3 is Y, X_4 is T, X_5 is E, X_6 is W, X_7 is L, X_8 is Y, X_9 is N, X_{10} is H, and X_{11} is absent); an intervening linker peptide (GGGSGGGSGGG (**SEQ ID NO:262**)); a VL Domain of a monoclonal antibody capable of binding to PD-1 (VL_{PD-1} hPD-1 mAb 7 VL2) (**SEQ ID NO:153**); an intervening linker peptide (**Linker 1:** GGGSGGGG (**SEQ ID NO:14**)); a VH Domain of a monoclonal antibody capable of binding PD-1 (VH_{PD-1} hPD-1 mAb 7 VH1) (**SEQ ID NO:147**); a cysteine-containing intervening linker peptide (**Linker 2:** GGCGGG (**SEQ ID NO:15**)); a Heterodimer-Promoting (E-coil) Domain (EVAALEK-EVAALEK-EVAALEK-EVAALEK (**SEQ ID NO:21**)); and a C-terminus.

[00353] The amino acid sequence of the third polypeptide chain of DART G is (**SEQ ID NO:279**):

QVQLVQSGAE VKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW
INTYTGESTY ADDFEGRFVF SMDTSASTAY LQISSLKAED TAVYYCARES
LYDYYSMDYW GQGTTVTVSS ASTKGPSVFP LAPSSKSTSG GTAALGCLVK
DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT
YICNVNHKPS NTKVDKRVEP KSCDKTHTCP PCPAPEAAGG PSVFLFPPKP
KDTLYITREP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN
STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ
VYTLPPSREE MTKNQVSLWC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV
LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGG
GGSGGGSGGG EIVLTQSPAT LSLSPGERAT LSCRASESVD NYGMSFMNWF
QQKPGQPPKL LIHAASNQGS GVPSRFSGSG SGTDFTLTIS SLEPEDFAVY
FCQQSKEVPY TFGGGTKVEI KGGGSGGGGQ VQLVQSGAEV KKPGASVKVS
CKASGYSFTS YWMNWVRQAP GQGLEWIGVI HPSDSETWLD QKFKDRVTIT
VDKSTSTAYM ELSSLRSEDT AVYYCAREHY GTSPFAYWGQ GTLVTVSSGG
CGGGEVAALE KEVAALEKEV AALEKEVAAL EK

[00354] The fourth polypeptide chain of DART G comprises, in the N-terminal to C-terminal direction: an N-terminus; a VL Domain of a monoclonal antibody capable of binding to PD-1 (VL_{PD-1} hPD-1 mAb 7 VL2) (**SEQ ID NO:153**); an intervening linker peptide (**Linker 1:** GGGSGGGG (**SEQ ID NO:14**)); a VH Domain of a monoclonal antibody capable of binding PD-1 (VH_{PD-1} hPD-1 mAb 7 VH1) (**SEQ ID NO:147**); a cysteine-containing intervening linker

peptide (**Linker 2:** GGCGGG (**SEQ ID NO:15**)); a Heterodimer-Promoting (K-coil) Domain (KVAALKE-KVAALKE-KVAALKE-KVAALKE (**SEQ ID NO:22**); and a C-terminus.

[00355] The amino acid sequence of the fourth polypeptide chains of DART G is (**SEQ ID NO:280**):

EIVLTQSPAT LSLSPGERAT LSCRASESVD NYGMSFMNWF QQKPGQPPKL
LIHAASNQGS GVPSRFSGSG SGTDFTLTIS SLEPEDFAVY FCQQSKEVPY
TFGGGTKVEI KGGGSGGGGQ VQLVQSGAEV KKPGASVKVS CKASGYSFTS
YWMNWVRQAP GQGLEWIGVI HPSDSETWLD QKFKDRVTIT VDKSTSTAYM
ELSSLRSEDT AVYYCAREHY GTSPFAYWGQ GTLVTVSSGG CGGGKVAALK
EKVAALKEKV AALKEKVAAL KE

E. Exemplary Three Chain Fc Region-Containing Diabody Having E/K-Coils

[00356] The present invention additionally provides PD-1 X LAG-3 bispecific, three chain Fc Region-containing diabodiesy comprising E/K-coil Heterodimer-Promoting Domains. An exemplary PD-1 X LAG-3 bispecific, three chain Fc Region-containing diabody comprising E/K-coil Heterodimer-Promoting Domains designated "**DART H**" was generated. The structure of this Fc Region-containing diabodies is detailed below. This exemplary PD-1 x LAG-3 diabody is intended to illustrate, but in no way limit, the scope of the invention.

[00357] DART H is a bispecific, three chain, Fc Region-containing diabody having one binding site specific for PD-1, one binding site specific for LAG-3, a variant knob/hole-bearing IgG1 Fc Region engineered for reduced FcγR binding, and E/K-coil Heterodimer-Promoting Domains.

[00358] The first polypeptide chain of DART H comprises, in the N-terminal to C-terminal direction: an N-terminus; a VL Domain of a monoclonal antibody capable of binding to PD-1 (VL_{PD-1} hPD-1 mAb 7 VL2) (**SEQ ID NO:153**); an intervening linker peptide (**Linker 1:** GGGSGGGG (**SEQ ID NO:14**)); a VH Domain of a monoclonal antibody capable of binding to LAG-3 (VH_{LAG-3} hLAG-3 mAb 1 VH1) (**SEQ ID NO:49**); a cysteine-containing intervening linker peptide (**Linker 2:** GGCGGG (**SEQ ID NO:15**)); a Heterodimer-Promoting (E-coil) Domain (EVAALEK-EVAALEK-EVAALEK-EVAALEK (**SEQ ID NO:21**)); an intervening linker (**Spacer-Linker 3:** GGGDKTHTCPPCP (**SEQ ID NO:263**)); a knob-bearing IgG1 CH2-CH3 Domain comprising substitutions L234A/L235A and having the C-terminal lysine residue (**SEQ ID NO:6**); and a C-terminus.

[00359] The amino acid sequence of the first polypeptide chain of DART H is (**SEQ ID NO:281**):

EIVLTQSPAT LSLSPGERAT LSCRASESVD NYGMSFMNWF QQKPGQPPKL
LIHAASNQGS GVPSRFSGSG SGTDFTLTIS SLEPEDFAVY FCQQSKEVPY
TFGGGTKVEI KGGGSGGGGQ VQLVQSGAEV KKPGASVKVS CKASGYTFTN
YGMNWVRQAP GQGLEWMGWI NTYTGESTYA DDFEGRFVFS MDTSASTAYL
QISSLKAEDT AVYYCARESL YDYYSMDYWG QGTTVTVSSG GCGGGEVAAL
EKEVAALEKE VAALEKEVAA LEKGGGDKTH TCPPCPAPEA AGGPSVFLFP
PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE
QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
EPQVYTLPPS REEMTKNQVS LWCLVKGFYP SDIAVEWESN GQPENNYKTT
PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS
PGK

[00360] The second polypeptide chain of DART H comprises, in the N-terminal to C-terminal direction: an N-terminus; a VL Domain of a monoclonal antibody capable of binding to LAG-3 (VL_{LAG-3} hLAG-3 mAb 1 VL4) (**SEQ ID NO:54**); an intervening linker peptide (**Linker 1:** GGGSGGGG (**SEQ ID NO:14**)); a VH Domain of a monoclonal antibody capable of binding to PD-1 (VH_{PD-1} hPD-1 mAb 7 VH1) (**SEQ ID NO:147**); a cysteine-containing intervening linker peptide (**Linker 2:** GGCGGG (**SEQ ID NO:15**)); a Heterodimer-Promoting (K-coil) Domain (KVAALKE-KVAALKE-KVAALKE-KVAALKE (**SEQ ID NO:22**)); and a C-terminus.

[00361] The amino acid sequence of the second polypeptide chain of DART H is (**SEQ ID NO:282**):

DIVMTQTPLS LSVTPGQPAS ISCKSSQSLL HSDAKTYLNW LLQKPGQPPE
RLIYLVSELD SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCWQGTHFP
YTFGGGTKVE IKGGGSGGGG QVQLVQSGAE VKKPGASVKV SCKASGYSFT
SYWMNWVRQA PGQGLEWIGV IHPSDSETWL DQKFKDRVTI TVDKSTSTAY
MELSSLRSED TAVYYCAREH YGTSPFAYWG QGTLVTVSSG GCGGGKVAAL
KEKVAALKEK VAALKEKVAA LKE

[00362] The third polypeptide chain of DART H comprises, in the N-terminal to C-terminal direction: an N-terminus; a hinge region (DKTHTCPPCP (**SEQ ID NO:31**); a hole-bearing IgG1 CH2-CH3 Domain comprising substitutions L234A/L235A and having the C-terminal lysine residue (**SEQ ID NO:7**); and a C-terminus.

[00363] The amino acid sequence of the third polypeptide chain of DART H is (**SEQ ID NO:283**):

DKTHTCPPCP APEAAGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED
PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK
CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLSCAVK
GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLVSKL TVDKSRWQQG
NVFSCSVMHE ALHNRYTQKS LSLSPGK

F. Exemplary Bispecific Antibody

[00364] An exemplary PD-1 X LAG-3 four chain bispecific antibody designated "**BSAB A**" was generated. The structure of this bispecific antibody is detailed below. This exemplary PD-1 x LAG-3 bispecific antibody is intended to illustrate, but in no way limit, the scope of the invention.

[00365] BSAB A is a bispecific antibody having one binding site specific for PD-1, one binding site specific for LAG-3, a variant IgG1 Fc Region engineered to reduce FcγR binding and to foster complexing between the two different heavy chain polypeptides (see, *e.g.*, WO 2011/143545).

[00366] The first polypeptide chain of BSAB A comprises, in the N-terminal to C-terminal direction: an N-terminus; a VH Domain of a monoclonal antibody capable of binding to PD-1 (VH_{PD-1} hPD-1 mAb 7 VH1) (**SEQ ID NO:147**); an IgG1 CH1 Domain (**SEQ ID NO:10**); a variant IgG1 hinge region comprising substitutions D221E/P228E (numbered by the EU index as in Kabat and underlined in **SEQ ID NO:286**, below); a variant IgG1 CH2-CH3 Domain comprising substitutions L234A/L235A/L368E (underlined in **SEQ ID NO:286**, below) and lacking the C-terminal residue; and a C-terminus.

[00367] The amino acid sequence of the first polypeptide chain of BSAB A is (**SEQ ID NO:286**):

QVQLVQSGAE VKKPGASVKV SCKASGYSFT SYWMNWVRQA PGQGLEWIGV
IHPSDSETWL DQKFKDRVTI TVDKSTSTAY MELSSLRSED TAVYYCAREH
YGTSPFAYWG QGTLVTVSSA STKGPSVFPL APSSKSTSGG TAALGCLVKD
YFPEPVTVSW NSGALTSGVH TFPAVLQSSG LYSLSSVVTV PSSSLGTQTY
ICNVNHKPSN TKVDKRVEPK SC**E**KTHTCP**E** CPAPE**AA**GGP SVFLFPPKPK
DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS
TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV
YTLPPSREEM TKNQVSLTC**E** VKGFYPSDIA VEWESNGQPE NNYKTTPPVL
DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPG

[00368] The second polypeptide chain of BSAB comprises, in the N-terminal to C-terminal direction: an N-terminus; a VL Domain of a monoclonal antibody capable of binding to PD-1

(VL_{PD-1} hPD-1 mAb 7 VL2) (**SEQ ID NO:153**); a Kappa CL Domain (**SEQ ID NO:8**), and a C-terminus.

[00369] The amino acid sequence of the second polypeptide chain of BSAB is (**SEQ ID NO:287**):

EIVLTQSPAT LSLSPGERAT LSCRASESVD NYGMSFMNWF QQKPGQPPKL
LIHAASNQGS GVPSRFSGSG SGTDFTLTIS SLEPEDFAVY FCQQSKEVPY
TFGGGTKVEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV
QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV
THQGLSSPVT KSFNRGEC

[00370] The third polypeptide chain of BSAB A comprises, in the N-terminal to C-terminal direction: an N-terminus; a VH Domain of a monoclonal antibody capable of binding to LAG-3 (VH_{LAG-3} hLAG-3 mAb 1 VH1) (**SEQ ID NO:49**); an IgG1 CH1 Domain (**SEQ ID NO:10**); a variant IgG1 hinge region comprising substitutions D221R/P228R (underlined in **SEQ ID NO:288**, below); a variant IgG1 CH2-CH3 Domain comprising substitutions L234A/L235A/L409R (underlined in **SEQ ID NO:288**, below) and lacking the C-terminal residue; and a C-terminus.

[00371] The amino acid sequence of the third polypeptide chain of BSAB A is (**SEQ ID NO:288**):

QVQLVQSGAE VKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW
INTYTGESTY ADDFEGRFVF SMDTSASTAY LQISSLKAED TAVYYCARES
LYDYYSMDYW GQGTTVTVSS ASTKGPSVFP LAPSSKSTSG GTAALGCLVK
DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT
YICNVNHKPS NTKVDKRVEP KSCRKTHTCP RCPAPEAAGG PSVFLFPPKP
KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN
STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ
VYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV
LDSDGSFFLY SRLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPG

[00372] The fourth polypeptide chain of BSAB A comprises, in the N-terminal to C-terminal direction: an N-terminus; a VL Domain of a monoclonal antibody capable of binding to LAG-3 (VL_{LAG-3} hLAG-3 mAb 1 VL4) (**SEQ ID NO:54**); a Kappa CL Domain (**SEQ ID NO:8**), and a C-terminus.

[00373] The amino acid sequence of the fourth polypeptide chain of BSAB A is (**SEQ ID NO:289**):

DIVMTQTPLS LSVTPGQPAS ISCKSSQSLL HSDAKTYLNW LLQKPGQPPE
RLIYLVSELD SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCWQGTHFP
YTFGGGTKVE IKRTVAAPSV FIFPPSDEQL KSGTASVVCL LNNFYPREAK
VQWKVDNALQ SGNSQESVTE QDSKDSTYSL SSTLTLSKAD YEKHKVYACE
VTHQGLSSPV TKSFNRGEC

IX. Reference Antibodies

A. Reference Anti-Human PD-1 Antibodies

[00374] In order to assess and characterize the novel anti-human PD-1-binding molecules of the present invention, the following reference antibodies were employed: nivolumab (also known as 5C4, BMS-936558, ONO-4538, MDX-1106, and marketed as OPDIVO® by Bristol-Myers Squibb), a human IgG4 antibody designated herein as "**PD-1 mAb A**;" and pembrolizumab (formerly known as lambrolizumab, also known as MK-3475, SCH-900475, and marketed as KEYTRUDA® by Merck) a humanized IgG4 antibody designated herein as "**PD-1 mAb B.**"

1. Nivolumab ("PD-1 mAb A")

[00375] The amino acid sequence of the Heavy Chain Variable Domain of **PD-1 mAb A** has the amino acid sequence (**SEQ ID NO:64**) (CDR_H residues are shown underlined):

QVQLVESGGG VVQPGRSLRL DCKASGITFS **NSGMH**WVRQA PGKGLEWVA**V**
IWYDGSKRYY **ADSVKG**RFTI SRDNSKNTLF LQMNSLRAED TAVYYCAT**ND**
DYWGQGTLVT VSS

[00376] The amino acid sequence of the Light Chain Variable Domain of **PD-1 mAb A** has the amino acid sequence (**SEQ** ID **NO:65**) (CDR_L residues are shown underlined):

EIVLTQSPAT LSLSPGERAT LSC**RASQSVS** **SYLA**WYQQKP GQAPRLLIY**D**
ASNRATGIPA RFSGSGSGTD FTLTISSLEP EDFAVYYC**QQ** **SSNWPRT**FGQ
GTKVEIK

2. Pembrolizumab ("PD-1 mAb B")

[00377] The amino acid sequence of the Heavy Chain Variable Domain of **PD-1 mAb B** has the amino acid sequence (**SEQ** ID **NO:66**) (CDR_H residues are shown underlined):

QVQLVQSGVE VKKPGASVKV SCKASGYTFT **NYYMY**WVRQA PGQGLEWMG**G**
INPSNGGTNF **NEKFKN**RVTL TTDSSTTTAY MELKSLQFDD TAVYYCAR**RD**
YRFDMGFDYW GQGTTVTVSS

[00378] The amino acid sequence of the Light Chain Variable Domain of **PD-1 mAb B** has the amino acid sequence (**SEQ ID NO:67**) (CDR_L residues are shown underlined):

EIVLTQSPAT LSLSPGERAT LSC**RASKGVS** **TSGYSYLH**WY QQKPGQAPRL LIY**LASYLES** GVPARFSGSG SGTDFTLTIS SLEPEDFAVY YC**QHSRDLPL** **T**FGGGTKVEIK

X. Methods of Production

[00379] An anti-human PD-1 polypeptide, and other PD-1 agonists, antagonists and modulators can be created from the polynucleotides and/or sequences of the anti-PD-1 antibodies PD-1 mAb 1-15 by methods known in the art, for example, synthetically or recombinantly. One method of producing such peptide agonists, antagonists and modulators involves chemical synthesis of the polypeptide, followed by treatment under oxidizing conditions appropriate to obtain the native conformation, that is, the correct disulfide bond linkages. This can be accomplished using methodologies well known to those skilled in the art (see, *e.g.*, Kelley, R. F. *et al.* (1990) In: GENETIC ENGINEERING PRINCIPLES AND METHODS, Setlow, J.K. Ed., Plenum Press, N.Y., vol. 12, pp 1-19; Stewart, J.M *et al.* (1984) SOLID PHASE PEPTIDE SYNTHESIS, Pierce Chemical Co., Rockford, IL; see also United States Patents Nos. 4,105,603; 3,972,859; 3,842,067; and 3,862,925).

[00380] Polypeptides of the invention may be conveniently prepared using solid phase peptide synthesis (Merrifield, B. (1986) "S*olid Phase Synthesis*," Science 232(4748):341-347; Houghten, R.A. (1985) "*General Method For The Rapid Solid-Phase Synthesis Of Large Numbers Of Peptides: Specificity Of Antigen-Antibody Interaction At The Level Of Individual Amino Acids*," Proc. Natl. Acad. Sci. (U.S.A.) 82(15):5131-5135; Ganesan, A. (2006) "*Solid-Phase Synthesis In The Twenty-First Century*," Mini Rev. Med. Chem. 6(1):3-10).

[00381] In yet another alternative, fully human antibodies having one or more of the CDRs of PD-1 mAb 1, PD-1 mAb 2, PD-1 mAb 3, PD-1 mAb 4, PD-1 mAb 5, PD-1 mAb 6, PD-1 mAb 7, PD-1 mAb 8, PD-1 mAb 9, PD-1 mAb 10, PD-1 mAb 11, PD-1 mAb 12, PD-1 mAb 13, PD-1 mAb 14, or PD-1 mAb 15, or which compete with PD-1 mAb 1, PD-1 mAb 2, PD-1 mAb 3, PD-1 mAb 4, PD-1 mAb 5, PD-1 mAb 6, PD-1 mAb 7, PD-1 mAb 8, PD-1 mAb 9, PD-1 mAb 10, PD-1 mAb 11, PD-1 mAb 12, PD-1 mAb 13, PD-1 mAb 14, or PD-1 mAb 15, for binding to human PD-1 or a soluble form thereof may be obtained through the use of commercially available mice that have been engineered to express specific human immunoglobulin proteins. Transgenic animals that are designed to produce a more desirable (*e.g.*, fully human antibodies) or more robust immune response may also be used for generation

of humanized or human antibodies. Examples of such technology are XENOMOUSE™ (Abgenix, Inc., Fremont, CA) and HUMAB-MOUSE® and TC MOUSE™ (both from Medarex, Inc., Princeton, NJ).

[00382] In an alternative, antibodies may be made recombinantly and expressed using any method known in the art. Antibodies may be made recombinantly by first isolating the antibodies made from host animals, obtaining the gene sequence, and using the gene sequence to express the antibody recombinantly in host cells (*e.g.*, CHO cells). Another method that may be employed is to express the antibody sequence in plants {*e.g.*, tobacco) or transgenic milk. Suitable methods for expressing antibodies recombinantly in plants or milk have been disclosed (see, for example, Peeters *et al.* (2001) "*Production Of Antibodies And Antibody Fragments In Plants*," Vaccine 19:2756; Lonberg, N. *et al.* (1995) "*Human Antibodies From Transgenic Mice*," Int. Rev. Immunol 13:65-93; and Pollock *et al.* (1999) "*Transgenic Milk As A Method For The Production Of Recombinant Antibodies*," J. Immunol Methods 231:147-157). Suitable methods for making derivatives of antibodies, *e.g.*, humanized, single-chain, *etc.* are known in the art. In another alternative, antibodies may be made recombinantly by phage display technology (see, for example, U.S. Patent Nos. 5,565,332; 5,580,717; 5,733,743; 6,265,150; and Winter, G. *et al.* (1994) "*Making Antibodies By Phage Display Technology*," Annu. Rev. Immunol. 12.433-455).

[00383] The antibodies or protein of interest may be subjected to sequencing by Edman degradation, which is well known to those of skill in the art. The peptide information generated from mass spectrometry or Edman degradation can be used to design probes or primers that are used to clone the protein of interest.

[00384] An alternative method of cloning the protein of interest is by "panning" using purified PD-1 or portions thereof for cells expressing an antibody or protein of interest that possesses one or more of the CDRs of PD-1 mAb 1, PD-1 mAb 2, PD-1 mAb 3, PD-1 mAb 4, PD-1 mAb 5, PD-1 mAb 6, PD-1 mAb 7, PD-1 mAb 8, PD-1 mAb 9, PD-1 mAb 10, PD-1 mAb 11, PD-1 mAb 12, PD-1 mAb 13, PD-1 mAb 14, or PD-1 mAb 15, or of an antibody that competes with PD-1 mAb 1, PD-1 mAb 2, PD-1 mAb 3, PD-1 mAb 4, PD-1 mAb 5, PD-1 mAb 6, PD-1 mAb 7, PD-1 mAb 8, PD-1 mAb 9, PD-1 mAb 10, PD-1 mAb 11, PD-1 mAb 12, PD-1 mAb 13, PD-1 mAb 14, or PD-1 mAb 15, for binding to human PD-1. The "panning" procedure may be conducted by obtaining a cDNA library from tissues or cells that express PD-1, overexpressing the cDNAs in a second cell type, and screening the transfected cells of

the second cell type for a specific binding to PD-1 in the presence or absence of PD-1 mAb 1, PD-1 mAb 2, PD-1 mAb 3, PD-1 mAb 4, PD-1 mAb 5, PD-1 mAb 6, PD-1 mAb 7, PD-1 mAb 8, PD-1 mAb 9, PD-1 mAb 10, PD-1 mAb 11, PD-1 mAb 12, PD-1 mAb 13, PD-1 mAb 14, or PD-1 mAb 15. Detailed descriptions of the methods used in cloning mammalian genes coding for cell surface proteins by "panning" can be found in the art (see, for example, Aruffo, A. *et al.* (1987) "*Molecular Cloning Of A CD28 cDNA By A High-Efficiency COS Cell Expression System*," Proc. Natl. Acad. Sci. (U.S.A.) 84:8573-8577 and Stephan, J. *et al.* (1999) "*Selective Cloning Of Cell Surface Proteins Involved In Organ Development: Epithelial Glycoprotein Is Involved In Normal Epithelial Differentiation*," Endocrinol. 140:5841-5854).

[00385] Vectors containing polynucleotides of interest can be introduced into the host cell by any of a number of appropriate means, including electroporation, transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE- dextran, or other substances; microprojectile bombardment; lipofection; and infection (*e.g.*, where the vector is an infectious agent such as vaccinia virus). The choice of introducing vectors or polynucleotides will often depend on features of the host cell.

[00386] Any host cell capable of overexpressing heterologous DNAs can be used for the purpose of isolating the genes encoding the antibody, polypeptide or protein of interest. Non-limiting examples of suitable mammalian host cells include but are not limited to COS, HeLa, and CHO cells. Preferably, the host cells express the cDNAs at a level of about 5-fold higher, more preferably 10-fold higher, even more preferably 20-fold higher than that of the corresponding endogenous antibody or protein of interest, if present, in the host cells. Screening the host cells for a specific binding to PD-1 is effected by an immunoassay or FACS. A cell overexpressing the antibody or protein of interest can be identified.

[00387] The invention includes polypeptides comprising an amino acid sequence of the antibodies of this invention. The polypeptides of this invention can be made by procedures known in the art. The polypeptides can be produced by proteolytic or other degradation of the antibodies, by recombinant methods (*i.e.*, single or fusion polypeptides) as described above or by chemical synthesis. Polypeptides of the antibodies, especially shorter polypeptides up to about 50 amino acids, are conveniently made by chemical synthesis. Methods of chemical synthesis are known in the art and are commercially available. For example, an anti-human PD-1 polypeptide could be produced by an automated polypeptide synthesizer employing the solid phase method.

[00388] The invention includes variants of PD-1 mAb 1, PD-1 mAb 2, PD-1 mAb 3, PD-1 mAb 4, PD-1 mAb 5, PD-1 mAb 6, PD-1 mAb 7, PD-1 mAb 8, PD-1 mAb 9, PD-1 mAb 10, PD-1 mAb 11, PD-1 mAb 12, PD-1 mAb 13, PD-1 mAb 14, or PD-1 mAb 15 antibodies and their polypeptide fragments that bind to PD-1, including functionally equivalent antibodies and fusion polypeptides that do not significantly affect the properties of such molecules as well as variants that have enhanced or decreased activity. Modification of polypeptides is routine practice in the art and need not be described in detail herein. Examples of modified polypeptides include polypeptides with conservative substitutions of amino acid residues, one or more deletions or additions of amino acids which do not significantly deleteriously change the functional activity, or use of chemical analogs. Amino acid residues that can be conservatively substituted for one another include but are not limited to: glycine/alanine; serine/threonine; valine/isoleucine/leucine; asparagine/glutamine; aspartic acid/glutamic acid; lysine/arginine; and phenylalanine/tyrosine. These polypeptides also include glycosylated and non-glycosylated polypeptides, as well as polypeptides with other post-translational modifications, such as, for example, glycosylation with different sugars, acetylation, and phosphorylation. Preferably, the amino acid substitutions would be conservative, *i.e.*, the substituted amino acid would possess similar chemical properties as that of the original amino acid. Such conservative substitutions are known in the art, and examples have been provided above. Amino acid modifications can range from changing or modifying one or more amino acids to complete redesign of a region, such as the Variable Domain. Changes in the Variable Domain can alter binding affinity and/or specificity. Other methods of modification include using coupling techniques known in the art, including, but not limited to, enzymatic means, oxidative substitution and chelation. Modifications can be used, for example, for attachment of labels for immunoassay, such as the attachment of radioactive moieties for radioimmunoassay. Modified polypeptides are made using established procedures in the art and can be screened using standard assays known in the art.

[00389] The invention encompasses fusion proteins comprising one or more of the polypeptides or PD-1 mAb 1, PD-1 mAb 2, PD-1 mAb 3, PD-1 mAb 4, PD-1 mAb 5, PD-1 mAb 6, PD-1 mAb 7, PD-1 mAb 8, PD-1 mAb 9, PD-1 mAb 10, PD-1 mAb 11, PD-1 mAb 12, PD-1 mAb 13, PD-1 mAb 14, or PD-1 mAb 15 antibodies of this invention. In one embodiment, a fusion polypeptide is provided that comprises a light chain, a heavy chain or both a light and heavy chain. In another embodiment, the fusion polypeptide contains a heterologous immunoglobulin constant region. In another embodiment, the fusion polypeptide

contains a Light Chain Variable Domain and a Heavy Chain Variable Domain of an antibody produced from a publicly-deposited hybridoma. For purposes of this invention, an antibody fusion protein contains one or more polypeptide domains that specifically bind to PD-1 and another amino acid sequence to which it is not attached in the native molecule, for example, a heterologous sequence or a homologous sequence from another region.

XI. Uses of the PD-1-Binding Molecules of the Present Invention

[00390] The present invention encompasses compositions, including pharmaceutical compositions, comprising the PD-1-binding molecules of the present invention (*e.g.*, anti-PD-1 antibodies, anti-PD-1 bispecific diabodies, *etc.*), polypeptides derived from such molecules, polynucleotides comprising sequences encoding such molecules or polypeptides, and other agents as described herein.

A. Thrapeutic Uses

[00391] As discussed above, PD-1 plays an important role in negatively regulating T-cell proliferation, function and homeostasis. Certain of the PD-1-binding molecules of the present invention have the ability to inhibit PD-1 function, and thus reverse the PD-1-mediated immune system inhibition. As such, PD-1 mAb 1, PD-1 mAb 3, PD-1 mAb 5, PD-1 mAb 6, PD-1 mAb 7, PD-1 mAb 8, PD-1 mAb 9, PD-1 mAb 10, PD-1 mAb 11, PD-1 mAb 12, PD-1 mAb 13, PD-1 mAb 14, and PD-1 mAb 15, their humanized derivatives, and molecules comprising their PD-1-binding fragments (*e.g.*, bispecific antibodies, bispecific diabodies (including, but not limited to, DART-A, DART-B, DART-C, DART-D, DART-E, DART-F, DART-G, DART-H, DART-I, and DART-J), *etc.*), or that compete for binding with such antibodies, may be used to block PD-1-mediated immune system inhibition, and thereby promote the activation of the immune system.

[00392] Such bispecific PD-1-binding molecules of the present invention that bind to PD-1 and another molecule involved in regulating an immune check point present on the cell surface (*e.g.*, LAG-3) augment the immune system by blocking immune system inhibition mediated by PD-1 and such immune check point molecules. Thus, such PD-1-binding molecules of the invention are useful for augmenting an immune response (*e.g.*, the T-cell mediated immune response) of a subject. In particular, such PD-1-binding molecules of the invention and may be used to treat any disease or condition associated with an undesirably suppressed immune

system, including cancer and diseases that are associated with the presence of a pathogen (*e.g.*, a bacterial, fungal, viral or protozoan infection).

[00393] The cancers that may be treated by such PD-1-binding molecules of the present invention include cancers characterized by the presence of a cancer cell selected from the group consisting of a cell of: an adrenal gland tumor, an AIDS-associated cancer, an alveolar soft part sarcoma, an astrocytic tumor, bladder cancer, bone cancer, a brain and spinal cord cancer, a metastatic brain tumor, a breast cancer, a carotid body tumors, a cervical cancer, a chondrosarcoma, a chordoma, a chromophobe renal cell carcinoma, a clear cell carcinoma, a colon cancer, a colorectal cancer, a cutaneous benign fibrous histiocytoma, a desmoplastic small round cell tumor, an ependymoma, a Ewing's tumor, an extraskeletal myxoid chondrosarcoma, a fibrogenesis imperfecta ossium, a fibrous dysplasia of the bone, a gallbladder or bile duct cancer, gastric cancer, a gestational trophoblastic disease, a germ cell tumor, a head and neck cancer, hepatocellular carcinoma, an islet cell tumor, a Kaposi's Sarcoma, a kidney cancer, a leukemia, a lipoma/benign lipomatous tumor, a liposarcoma/malignant lipomatous tumor, a liver cancer, a lymphoma, a lung cancer, a medulloblastoma, a melanoma, a meningioma, a multiple endocrine neoplasia, a multiple myeloma, a myelodysplastic syndrome, a neuroblastoma, a neuroendocrine tumors, an ovarian cancer, a pancreatic cancer, a papillary thyroid carcinoma, a parathyroid tumor, a pediatric cancer, a peripheral nerve sheath tumor, a phaeochromocytoma, a pituitary tumor, a prostate cancer, a posterious uveal melanoma, a rare hematologic disorder, a renal metastatic cancer, a rhabdoid tumor, a rhabdomysarcoma, a sarcoma, a skin cancer, a soft-tissue sarcoma, a squamous cell cancer, a stomach cancer, a synovial sarcoma, a testicular cancer, a thymic carcinoma, a thymoma, a thyroid metastatic cancer, and a uterine cancer.

[00394] In particular, such PD-1-binding molecules of the present invention may be used in the treatment of colorectal cancer, hepatocellular carcinoma, glioma, kidney cancer, breast cancer, multiple myeloma, bladder cancer, neuroblastoma; sarcoma, non-Hodgkin's lymphoma, non-small cell lung cancer, ovarian cancer, pancreatic cancer and rectal cancer.

[00395] Pathogen-associated diseases that may be treated by such PD-1-binding molecules of the present invention include chronic viral, bacterial, fungal and parasitic infections. Chronic infections that may be treated by the PD-1-binding molecules of the present invention include Epstein Barr virus, Hepatitis A Virus (HAV); Hepatitis B Virus (HBV); Hepatitis C Virus (HCV); herpes viruses (*e.g.* HSV-1, HSV-2, HHV-6, CMV), Human Immunodeficiency

Virus (HIV), Vesicular Stomatitis Virus (VSV), *Bacilli, Citrobacter, Cholera, Diphtheria, Enterobacter, Gonococci, Helicobacter pylori, Klebsiella, Legionella, Meningococci,* mycobacteria, *Pseudomonas, Pneumonococci,* rickettsia bacteria, *Salmonella, Serratia, Staphylococci, Streptococci, Tetanus, Aspergillus* (fumigatus, niger, *etc.*), *Blastomyces dermatitidis, Candida* (albicans, krusei, glabrata, tropicalis, *etc.*), *Cryptococcus neoformans,* Genus *Mucorales* (mucor, absidia, rhizopus), *Sporothrix schenkii, Paracoccidioides brasiliensis, Coccidioides immitis, Histoplasma capsulatum, Leptospirosis, Borrelia burgdorferi*, helminth parasite (hookworm, tapeworms, flukes, flatworms (*e.g. Schistosomia*), *Giardia lambia, trichinella, Dientamoeba Fragilis, Trypanosoma brucei, Trypanosoma cruzi,* and *Leishmania donovani*.

[00396] Such PD-1-binding molecules of the invention can be combined with other anti-cancer agents, in particular, molecules that specifically bind a cancer antigen (*e.g.*, antibodies, diabodies). Anti-cancer therapies that may be combined with the PD-1-binding molecules of the invention include molecules which specifically bind one more cancer antigens including: **19.9** as found in colon cancer, gastric cancer mucins; **4.2**; **A33** (a colorectal carcinoma antigen; Almqvist, Y. 2006, *Nucl Med Biol.* Nov;33(8):991-998); **ADAM-9** (United States Patent Publication No. 2006/0172350; PCT Publication No. WO 06/084075); **AH6** as found in gastric cancer; **ALCAM** (PCT Publication No. WO 03/093443); **APO-1 (malignant human lymphocyte antigen)** (Trauth *et al.* (1989) *"Monoclonal Antibody-Mediated Tumor Regression By Induction Of Apoptosis,"* Science 245:301-304); **B1** (Egloff, A.M. *et al.* 2006, *Cancer Res.* 66(1):6-9); **B7-H3** (Collins, M. *et al.* (2005) "*The B7 Family Of Immune-Regulatory Ligands*," Genome Biol. 6:223.1-223.7). Chapoval, A. *et al.* (2001) "*B7-H3: A Costimulatory Molecule For T Cell Activation and IFN-γ Production*," Nature Immunol. 2:269–274; Sun, M. *et al.* (2002) "*Characterization of Mouse and Human B7-H3 Genes*," J. Immunol. 168:6294-6297); **BAGE** (Bodey, B. 2002 *Expert Opin Biol Ther.* 2(6):577-84); **beta-catenin** (Prange W. *et al.* 2003 *J Pathol.* 201(2):250-9); **blood group ALe^b/Le^y** as found in colonic adenocarcinoma; **Burkitt's lymphoma antigen-38.13**, **C14** as found in colonic adenocarcinoma; **CA125 (ovarian carcinoma antigen)** (Bast, R.C. Jr. *et al.* 2005 *Int J Gynecol Cancer* 15 Suppl 3:274-81 ; Yu *et al.* (1991) *"Coexpression Of Different Antigenic Markers On Moieties That Bear CA 125 Determinants,"* Cancer Res. 51(2):468-475); **Carboxypeptidase M** (United States Patent Publication No. 2006/0166291); **CD5** (Calin, G.A. *et al.* 2006 *Semin Oncol.* 33(2):167-73; **CD19** (Ghetie *et al.* (1994) *"Anti-CD19 Inhibits The Growth Of Human B-Cell Tumor Lines In Vitro And Of Daudi Cells In SCID Mice By Inducing*

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142:3662-3667; Möller *et al.* (1991) *"Bi-specific-Monoclonal-Antibody-Directed Lysis Of Ovarian Carcinoma Cells By Activated Human T Lymphocytes,"* Cancer Immunol. Immunother. 33(4):210-216; Ragupathi, G. 2005 *Cancer Treat Res.* 123:157-80); **L6** and **L20** (human lung carcinoma antigens; Hellström *et al.* (1986) *"Monoclonal Mouse Antibodies Raised Against Human Lung Carcinoma,"* Cancer Res. 46:3917-3923); **LEA**; **LUCA-2** (United States Patent Publication No. 2006/0172349; PCT Publication No. WO 06/083852); **M1:22:25:8**; **M18**; **M39**; **MAGE** (MAGE-1; MAGE-3; (Bodey, B. 2002 *Expert Opin Biol Ther.* 2(6):577-84); **MART** (Kounalakis, N. *et al.* 2005 *Curr Oncol Rep.* 7(5):377-82; **mesothelin** (Chang K, and Pastan I. 1996 "*Molecular cloning of mesothelin, a differentiation antigen present on mesothelium, mesotheliomas, and ovarian cancers*," Proc Natl Acad Sci USA 93:136–40), **MUC-1** (Mathelin, C. 2006 *Gynecol Obstet Fertil.* 34(7-8):638-46); **MUM-1** (Castelli, C. *et al.* 2000 *J Cell Physiol.* 182(3):323-31); **Myl**; **N-acetylglucosaminyltransferase** (Dennis, J.W. 1999 *Biochim Biophys Acta.* 6;1473(1):21-34); **neoglycoprotein**; **NS-10** as found in adenocarcinomas; **OFA-1**; **OFA-2**; **Oncostatin M** (Oncostatin Receptor Beta; United States Patent No. 7,572,896; PCT Publication No. WO 06/084092); **p15** (Gil, J. *et al.* 2006 *Nat Rev Mol Cell Biol.* 7(9):667-77); **p97** (melanoma-associated antigen; Estin *et al.* (1989) *"Transfected Mouse Melanoma Lines That Express Various Levels Of Human Melanoma-Associated Antigen p97,"* J. Natl. Cancer Instit. 81(6):445-454); **PEM** (polymorphic epithelial mucin; Hilkens *et al.* (1992) *"Cell Membrane-Associated Mucins And Their Adhesion-Modulating Property,"* Trends in Biochem. Sci. 17:359-363); **PEMA (polymorphic epithelial mucin antigen)**; **PIPA** (United States Patent No. 7,405,061; PCT Publication No. WO 04/043239); **PSA** (prostate-specific antigen; Henttu *et al.* (1989) *"cDNA Coding For The Entire Human Prostate Specific Antigen Shows High Homologies To The Human Tissue Kallikrein Genes,"* Biochem. Biophys. Res. Comm. 10(2):903-910; Israeli *et al.* (1993) *"Molecular Cloning Of A Complementary DNA Encoding A Prostate-Specific Membrane Antigen,"* Cancer Res. 53:227-230; Cracco, C.M. *et al.* 2005 *Minerva Urol Nefrol.* 57(4):301-11); **PSMA** (prostate-specific membrane antigen; Ragupathi, G. 2005 *Cancer Treat Res.* 123:157-180); **prostatic acid phosphate** (Tailor *et al.* (1990) *"Nucleotide Sequence Of Human Prostatic Acid Phosphatase Determined From A Full-Length cDNA Clone,"* Nucl. Acids Res. 18(16):4928); **R_{24}** as found in melanoma; **ROR1** (United States Patent No. 5,843,749); **sphingolipids**; **SSEA-1**; **SSEA-3**; **SSEA-4**; **sTn** (Holmberg, L.A. 2001 *Expert Opin Biol Ther.* 1(5):881-91); **T cell receptor derived peptide** from a cutaneous T cell lymphoma (*see* Edelson (1998) *"Cutaneous T-Cell Lymphoma: A Model For Selective Immunotherapy,"* Cancer J Sci Am. 4:62-71); **T_5A_7** found in myeloid cells; **TAG-72**

(Yokota *et al.* (1992) *"Rapid Tumor Penetration Of A Single-Chain Fv And Comparison With Other Immunoglobulin Forms,"* Cancer Res. 52:3402-3408); **TL5** (blood group A); **TNF-receptor** (TNF-α receptor, TNF-ß receptor; **TNF-γ receptor** (van Horssen, R. *et al.* 2006 *Oncologist.* 11(4):397-408; Gardnerova, M. *et al.* 2000 *Curr Drug Targets.* 1(4):327-64); **TRA-1-85** (blood group H); **Transferrin Receptor** (United States Patent No. 7,572,895; PCT Publication No. WO 05/121179); **5T4** (TPBG, trophoblast glycoprotein; Boghaert, E.R. *et al.* (2008) "*The Oncofetal Protein,* 5T4, *Is A Suitable Target For Antibody-Guided Anti-Cancer Chemotherapy With Calicheamicin*," Int. J. Oncol. 32(1):221-234; Eisen, T. *et al.* (2014) "*Naptumomab Estafenatox: Targeted Immunotherapy with a Novel Immunotoxin*," Curr. Oncol. Rep. 16:370, pp. 1-6**); TSTA (tumor-specific transplantation antigen)** such as virally-induced tumor antigens including T-antigen DNA tumor viruses and envelope antigens of RNA tumor viruses, oncofetal antigen-alpha-fetoprotein such as CEA of colon, bladder tumor oncofetal antigen (Hellström *et al.* (1985) *"Monoclonal Antibodies To Cell Surface Antigens Shared By Chemically Induced Mouse Bladder Carcinomas,"* Cancer. Res. 45:2210-2188); **VEGF** (Pietrantonio, F. *et al.* (2015) "*Bevacizumab-Based Neoadjuvant Chemotherapy For Colorectal Cancer Liver Metastases: Pitfalls And Helpful Tricks In A Review For Clinicians*," Crit. Rev. Oncol. Hematol. 95(3):272-281; Grabowski, J.P. (2015) "*Current Management Of Ovarian Cancer*," Minerva Med. 106(3):151-156; Field, K.M. (2015) "*Bevacizumab And Glioblastoma: Scientific Review, Newly Reported Updates, And Ongoing Controversies*," Cancer 121(7):997-1007; Suh, D.H. *et al.* (2015) "*Major Clinical Research Advances In Gynecologic Cancer In 2014*," J. Gynecol. Oncol. 26(2):156-167; Liu, K.J. *et al.* (2015) "*Bevacizumab In Combination With Anticancer Drugs For Previously Treated Advanced Non-Small Cell Lung Cancer*," Tumour Biol. 36(3):1323-1327; Di Bartolomeo, M. *et al.* (2015) "Bevacizumab treatment in the elderly patient with metastatic colorectal cancer," Clin. Interv. Aging 10:127-133); **VEGF Receptor** (O'Dwyer. P.J. 2006 *Oncologist.* 11(9):992-998); **VEP8**; **VEP9**; **VIM-D5**; and **Y hapten**, **Le^y** as found in embryonal carcinoma cells.

[00397] In certain embodiments, such anti-PD-1-binding molecules of the invention are used in combination with one or more molecules that specifically bind 5T4, B7H3, CD19, CD20, CD51, CD123, DR5, EGFR, EpCam, GD2, gpA33, HER2, ROR-1, TAG-72, VEGF-A antibody, and/or VEGFR2.

[00398] Such PD-1-binding molecules of the invention can be combined with an immunogenic agent such as a tumor vaccine. Such vaccines may comprise purified tumor

antigens (including recombinant proteins, peptides, and carbohydrate molecules), autologous or allogeneic tumor cells. A number of tumor vaccine strategies have been described (see for example, Palena, C., *et al.*, (2006) "*Cancer vaccines: preclinical studies and novel strategies*," Adv. Cancer Res. 95, 115-145; Mellman, I., *et al.* (2011) "*Cancer immunotherapy comes of age*," Nature 480, 480–489; Zhang, X. M. *et al.* (2008) "*The anti-tumor immune response induced by a combination of MAGE-3/MAGE-n-derived peptides*," Oncol. Rep. 20, 245–252; Disis, M. L. *et al.* (2002) "*Generation of T-cell immunity to the HER-2/neu protein after active immunization with HER-2/neu peptide-based vaccines*," J. Clin. Oncol. 20, 2624–2632; Vermeij, R. *et al.* (2012) "*Potentiation of a p53-SLP vaccine by cyclophosphamide in ovarian cancer: a single-arm phase II study*." Int. J. Cancer 131, E670–E680). Such PD-1-binding molecules of the invention can be combined with chemotherapeutic regimes. In these instances, it may be possible to reduce the dose of chemotherapeutic reagent administered (Mokyr *et al.* (1998) Cancer Research 58: 5301-5304).

[00399] Such PD-1-binding molecules of the invention can be combined with other immunostimulatory molecules such as antibodies which activate host immune responsiveness to provide for increased levels of T-cell activation. In particular, anti-PD-1 antibodies, anti-PD-L1 antibodies and/or an anti-CTLA-4 antibodies have been demonstrated to active the immune system (see, *e.g.*, del Rio, M-L. *et al.* (2005) "*Antibody-Mediated Signaling Through PD-1 Costimulates T Cells And Enhances CD28-Dependent Proliferation*," Eur. J. Immunol 35:3545-3560; Barber, D. L. *et al.* (2006) "*Restoring function in exhausted CD8 T cells during chronic viral infection*," Nature 439, 682–687; Iwai, Y. *et al.* (2002) "*Involvement Of PD-L1 On Tumor Cells In The Escape From Host Immune System And Tumor Immunotherapy By PD-L1 Blockade*," Proc. Natl Acad. Sci. USA 99, 12293–12297; Leach, D. R., *et al.*, (1996) "*Enhancement Of Antitumor Immunity By CTLA-4 Blockade*," Science 271, 1734-1736). Additional immunostimulatory molecules that may be combined with the PD-1-binding molecules of the invention include antibodies to molecules on the surface of dendritic cells that activate dendritic cell (DC) function and antigen presentation, anti-CD40 antibodies able to substitute for T-cell helper activity, and activating antibodies to T-cell costimulatory molecules such as PD-L1, CTLA-4, OX-40 4-1BB, and ICOS (see, for example, Ito *et al.* (2000) "*Effective Priming Of Cytotoxic T Lymphocyte Precursors By Subcutaneous Administration Of Peptide Antigens In Liposomes Accompanied By Anti-CD40 And Anti-CTLA-4 Antibodies*,"Immunobiology 201:527-40; U.S. Pat. No. 5,811,097; Weinberg *et al.* (2000) "*Engagement of the OX-40 Receptor In Vivo Enhances Antitumor Immunity*," Immunol

164:2160-2169; Melero *et al.* (1997) "*Monoclonal Antibodies Against The 4-1BB T-Cell Activation Molecule Eradicate Established Tumors*," Nature Medicine 3: 682-685; Hutloff *et al.* (1999) "*ICOS Is An Inducible T-Cell Co-Stimulator Structurally And Functionally Related To CD28*," Nature 397: 263-266; and Moran, A.E. *et al.* (2013) "*The TNFRs OX40, 4-1BB, and CD40 As Targets For Cancer Immunotherapy*," Curr Opin Immunol. 2013 Apr; 25(2): 10.1016/j.coi.2013.01.004), and/or stimulatory Chimeric Antigen Receptors (**CARs**) comprising an antigen binding domain directed against a disease antigen fused to one or more intracellular signaling domains from various costimulatory protein receptors (*e.g.*, CD28, 4-1BB, ICOS, OX40, *etc.*) which serve to stimulate T-cells upon antigen binding (see, for example, Tettamanti, S. *et al.* (2013) "*Targeting Of Acute Myeloid Leukaemia By Cytokine-Induced Killer Cells Redirected With A Novel CD123-Specific Chimeric* Antigen *Receptor*," Br. J. Haematol. 161:389-401; Gill, S. *et al.* (2014) "*Efficacy Against Human Acute Myeloid Leukemia And Myeloablation Of Normal Hematopoiesis In A Mouse Model Using Chimeric Antigen Receptor-Modified T Cells*," Blood 123(15): 2343-2354; Mardiros, A. *et al.* (2013) "*T Cells Expressing CD123-Specific Chimeric Antigen Receptors Exhibit Specific Cytolytic Effector Functions And Antitumor Effects Against Human Acute Myeloid Leukemia*," Blood 122:3138-3148; Pizzitola, I. *et al.* (2014) "*Chimeric Antigen Receptors Against CD33/CD123 Antigens Efficiently Target Primary Acute Myeloid Leukemia Cells in vivo*," Leukemia doi:10.1038/leu.2014.62).

[00400] Such PD-1-binding molecules of the invention can be combined with inhibitory Chimeric Antigen Receptors (**iCARs**) to divert off target immunotherapy responses. iCARs an antigen binding domain directed against a disease antigen fused to one or more intracellular signaling domains from various inhibitory protein receptors (*e.g.*, CTLA-4, PD-1, *etc.*) which serve to constrain T-cell responses upon antigen binding (see, for example, Fedorov V.D. (2013) "*PD-1– and CTLA-4–Based Inhibitory Chimeric Antigen Receptors (iCARs) Divert Off-Target Immunotherapy Responses*," Sci. Tranl. Med. 5:215ra172 doi:10.1126/ scitranslmed.3006597.

[00401] In particular, such anti-PD-1-binding molecules of the invention are used in combination with an anti-CD137 antibody, an anti-CTLA-4 antibody, an anti-OX40 antibody, an anti-LAG-3 antibody, an anti-PD-L1 antibody, an anti-TIGIT antibody, an anti TIM-3 antibody and/or a cancer vaccine.

B. Diagnostic and Theranostic Utility

[00402] Certain of the PD-1-binding molecules of the present invention exhibit little of no ability to block binding between PD-1 and the PD-1L ligand. As such, antibodies PD-1 mAb 2 and PD-1 mAb 4, their humanized derivatives, and molecules comprising their PD-1-binding fragments (*e.g.*, bispecific diabodies, *etc.*) or that compete for binding with such antibodies may be detectably labeled (*e.g.*, with radioactive, enzymatic, fluorescent, chemiluminescent, paramagnetic, diamagnetic, or other labelling moieties) and used in the detection of PD-1 in samples or in the imaging of PD-1 on cells. Since such molecules do not affect the biological activity of PD-1, they are particularly useful in methods of determining the extent, location and change in PD-1 expression in subjects (*e.g.*, subjects being treated for cancer associated with the expression or targeting of PD-1).

XII. Pharmaceutical Compositions

[00403] The compositions of the invention include bulk drug compositions useful in the manufacture of pharmaceutical compositions (*e.g.*, impure or non-sterile compositions) and pharmaceutical compositions (*i.e.*, compositions that are suitable for administration to a subject or patient) that can be used in the preparation of unit dosage forms. Such compositions comprise a prophylactically or therapeutically effective amount of the PD-1-binding molecules of the present invention, or a combination of such agents and a pharmaceutically acceptable carrier. Preferably, compositions of the invention comprise a prophylactically or therapeutically effective amount of the PD-1-binding molecules of the present invention and a pharmaceutically acceptable carrier. The invention particularly encompasses such pharmaceutical compositions in which the PD-1-binding molecule is: a PD-1 mAb 1, PD-1 mAb 2, PD-1 mAb 3, PD-1 mAb 4, PD-1 mAb 5, PD-1 mAb 6, PD-1 mAb 7, PD-1 mAb 8, PD-1 mAb 9, PD-1 mAb 10, PD-1 mAb 11, PD-1 mAb 12, PD-1 mAb 13, PD-1 mAb 14, or PD-1 mAb 15; a humanized PD-1 mAb 1; PD-1 mAb 2, PD-1 mAb 3, PD-1 mAb 4, PD-1 mAb 5, PD-1 mAb 6, PD-1 mAb 7, PD-1 mAb 8, PD-1 mAb 9, PD-1 mAb 10, PD-1 mAb 11, PD-1 mAb 12, PD-1 mAb 13, PD-1 mAb 14, or PD-1 mAb 15; a PD-1-binding fragment of any such antibody; or in which the PD-1-binding molecule is a bispecific PD-1 diabody (*e.g.*, a PD-1 x LAG-3 bispecific diabody). Especially encompassed are such molecules that comprise the 3 CDR_{L}s and the 3 CDR_{H}s of PD-1 mAb 1, PD-1 mAb 2, PD-1 mAb 3, PD-1 mAb 4, PD-1 mAb 5, PD-1 mAb 6, PD-1 mAb 7, PD-1 mAb 8, PD-1 mAb 9, PD-1 mAb 10, PD-1 mAb 11, PD-1 mAb 12, PD-1 mAb 13, PD-1 mAb 14, or PD-1 mAb 15 antibody; a humanized PD-1

mAb 1; PD-1 mAb 2, PD-1 mAb 3, PD-1 mAb 4, PD-1 mAb 5, PD-1 mAb 6, PD-1 mAb 7, PD-1 mAb 8, PD-1 mAb 9, PD-1 mAb 10, PD-1 mAb 11, PD-1 mAb 12, PD-1 mAb 13, PD-1 mAb 14, or PD-1 mAb 15.

[00404] The invention also encompasses such pharmaceutical compositions that additionally include a second therapeutic antibody (*e.g.*, tumor-specific monoclonal antibody) that is specific for a particular cancer antigen, and a pharmaceutically acceptable carrier.

[00405] In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant (*e.g.*, Freund's adjuvant (complete and incomplete), excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like.

[00406] Generally, the ingredients of compositions of the invention are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[00407] The compositions of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include, but are not limited to those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, *etc.*, and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, *etc.*

[00408] The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with a PD-1-binding molecule of the present invention (and more preferably, a PD-1 mAb 1, PD-1 mAb 2, PD-1 mAb 3, PD-1 mAb 4, PD-1 mAb 5, PD-1 mAb 6, PD-1 mAb 7, PD-1 mAb 8, PD-1 mAb 9, PD-1 mAb 10, PD-1 mAb 11, PD-1 mAb 12, PD-1 mAb 13, PD-1 mAb 14, or PD-1 mAb 15; a humanized PD-1 mAb 1, PD-1 mAb 2, PD-1 mAb 3, PD-1 mAb 4, PD-1 mAb 5, PD-1 mAb 6, PD-1 mAb 7, PD-1 mAb 8, PD-1 mAb 9, PD-1 mAb 10, PD-1 mAb 11, PD-1 mAb 12, PD-1 mAb 13, PD-1 mAb 14, or PD-1 mAb 15 antibody; a PD-1-binding fragment of any such antibody; or in which the PD-1-binding molecule is a bispecific PD-1 diabody (*e.g.*, a PD-1 x LAG-3 bispecific diabody)). Especially encompassed are such molecules that comprise the 3 CDR_{LS} and the 3 CDR_{HS} of PD-1 mAb 1, PD-1 mAb 2, PD-1 mAb 3, PD-1 mAb 4, PD-1 mAb 5, PD-1 mAb 6, PD-1 mAb 7, PD-1 mAb 8, PD-1 mAb 9, PD-1 mAb 10, PD-1 mAb 11, PD-1 mAb 12, PD-1 mAb 13, PD-1 mAb 14, or PD-1 mAb 15, alone or with such pharmaceutically acceptable carrier. Additionally, one or more other prophylactic or therapeutic agents useful for the treatment of a disease can also be included in the pharmaceutical pack or kit. The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

[00409] The present invention provides kits that can be used in the above methods. A kit can comprise any of the PD-1-binding molecules of the present invention. The kit can further comprise one or more other prophylactic and/or therapeutic agents useful for the treatment of cancer, in one or more containers; and/or the kit can further comprise one or more cytotoxic antibodies that bind one or more cancer antigens associated with cancer. In certain embodiments, the other prophylactic or therapeutic agent is a chemotherapeutic. In other embodiments, the prophylactic or therapeutic agent is a biological or hormonal therapeutic.

XIII. Methods of Administration

[00410] The compositions of the present invention may be provided for the treatment, prophylaxis, and amelioration of one or more symptoms associated with a disease, disorder or infection by administering to a subject an effective amount of a fusion protein or a conjugated molecule of the invention, or a pharmaceutical composition comprising a fusion protein or a conjugated molecule of the invention. In a preferred aspect, such compositions are substantially purified (*i.e.*, substantially free from substances that limit its effect or produce undesired side effects). In a specific embodiment, the subject is an animal, preferably a mammal such as non-primate (*e.g.*, bovine, equine, feline, canine, rodent, *etc.*) or a primate (*e.g.*, monkey such as, a cynomolgus monkey, human, *etc.*). In a preferred embodiment, the subject is a human.

[00411] Various delivery systems are known and can be used to administer the compositions of the invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the antibody or fusion protein, receptor-mediated endocytosis (See, *e.g.*, Wu *et al.* (1987) *"Receptor-Mediated In Vitro Gene Transformation By A Soluble DNA Carrier System,"* J. Biol. Chem. 262:4429-4432), construction of a nucleic acid as part of a retroviral or other vector, *etc.*

[00412] Methods of administering a molecule of the invention include, but are not limited to, parenteral administration (*e.g.*, intradermal, intramuscular, intraperitoneal, intravenous and subcutaneous), epidural, and mucosal (*e.g.*, intranasal and oral routes). In a specific embodiment, the PD-1-binding molecules of the present invention are administered intramuscularly, intravenously, or subcutaneously. The compositions may be administered by any convenient route, for example, by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, *etc.*) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent. See, *e.g.*, U.S. Patent Nos. 6,019,968; 5,985, 320; 5,985,309; 5,934,272; 5,874,064; 5,855,913; 5,290,540; and 4,880,078; and PCT Publication Nos. WO 92/19244; WO 97/32572; WO 97/44013; WO 98/31346; and WO 99/66903, each of which is incorporated herein by reference in its entirety.

[00413] The invention also provides that the PD-1-binding molecules of the present invention are packaged in a hermetically sealed container such as an ampoule or sachette

indicating the quantity of the molecule. In one embodiment, such molecules are supplied as a dry sterilized lyophilized powder or water free concentrate in a hermetically sealed container and can be reconstituted, *e.g.*, with water or saline to the appropriate concentration for administration to a subject. Preferably, the PD-1-binding molecules of the present invention are supplied as a dry sterile lyophilized powder in a hermetically sealed container.

[00414] The lyophilized PD-1-binding molecules of the present invention should be stored at between 2°C and 8°C in their original container and the molecules should be administered within 12 hours, preferably within 6 hours, within 5 hours, within 3 hours, or within 1 hour after being reconstituted. In an alternative embodiment, such molecules are supplied in liquid form in a hermetically sealed container indicating the quantity and concentration of the molecule, fusion protein, or conjugated molecule. Preferably, such PD-1-binding molecules when provided in liquid form are supplied in a hermetically sealed container.

[00415] The amount of the composition of the invention which will be effective in the treatment, prevention or amelioration of one or more symptoms associated with a disorder can be determined by standard clinical techniques. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the condition, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

[00416] As used herein, an "**effective amount**" of a pharmaceutical composition, in one embodiment, is an amount sufficient to effect beneficial or desired results including, without limitation, clinical results such as decreasing symptoms resulting from the disease, attenuating a symptom of infection (*e.g.*, viral load, fever, pain, sepsis, *etc.*) or a symptom of cancer (*e.g.*, the proliferation, of cancer cells, tumor presence, tumor metastases, *etc.*), thereby increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, enhancing the effect of another medication such as via targeting and/or internalization, delaying the progression of the disease, and/ or prolonging survival of individuals.

[00417] An effective amount can be administered in one or more administrations. For purposes of this invention, an effective amount of drug, compound, or pharmaceutical composition is an amount sufficient to reduce the proliferation of (or the effect of) viral

presence and to reduce and /or delay the development of the viral disease, either directly or indirectly. In some embodiments, an effective amount of a drug, compound, or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition. Thus, an "effective amount" may be considered in the context of administering one or more chemotherapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved. While individual needs vary, determination of optimal ranges of effective amounts of each component is within the skill of the art.

[00418] For the PD-1-binding molecules encompassed by the invention, the dosage administered to a patient is preferably determined based upon the body weight (kg) of the recipient subject. For the PD-1-binding molecules encompassed by the invention, the dosage administered to a patient is typically at least about 0.01 μg/kg, at least about 0.05 μg/kg, at least about 0.1 μg/kg, at least about 0.2 μg/kg, at least about 0.5 μg/kg, at least about 1 μg/kg, at least about 2 μg/kg, at least about 5 μg/kg, at least about 10 μg/kg, at least about 20 μg/kg, at least about 50 μg/kg, at least about 0.1 mg/kg, at least about 1 mg/kg, at least about 3 mg/kg, at least about 5 mg/kg, at least about 10 mg/kg, at least about 30 mg/kg, at least about 50 mg/kg, at least about 75 mg/kg, at least about 100 mg/kg, at least about 125 mg/kg, at least about 150 mg/kg or more of the subject's body weight.

[00419] The dosage and frequency of administration of a PD-1-binding molecule of the present invention may be reduced or altered by enhancing uptake and tissue penetration of the molecule by modifications such as, for example, lipidation.

[00420] The dosage of a PD-1-binding molecule of the invention administered to a patient may be calculated for use as a single agent therapy. Alternatively, the molecule may be used in combination with other therapeutic compositions and the dosage administered to a patient are lower than when said molecules are used as a single agent therapy.

[00421] The pharmaceutical compositions of the invention may be administered locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion, by injection, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a molecule of the invention, care must be taken to use materials to which the molecule does not absorb.

[00422] The compositions of the invention can be delivered in a vesicle, in particular a liposome (*See* Langer (1990) *"New Methods Of Drug Delivery,"* Science 249:1527-1533); Treat *et al.*, in LIPOSOMES IN THE THERAPY OF INFECTIOUS DISEASE AND CANCER, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, ibid., pp. 3 17-327).

[00423] The compositions of the invention can be delivered in a controlled-release or sustained-release system. Any technique known to one of skill in the art can be used to produce sustained-release formulations comprising one or more of the PD-1-binding molecule(s) of the invention. *See, e.g.*, U.S. Patent No. 4,526,938; PCT publication WO 91/05548; PCT publication WO 96/20698; Ning *et al.* (1996) *"Intratumoral Radioimmunotheraphy Of A Human Colon Cancer Xenograft Using A Sustained-Release Gel,"* Radiotherapy & Oncology 39:179-189, Song *et al.* (1995) *"Antibody Mediated Lung Targeting Of Long-Circulating Emulsions,"* PDA Journal of Pharmaceutical Science & Technology 50:372-397; Cleek *et al.* (1997) *"Biodegradable Polymeric Carriers For A bFGF Antibody For Cardiovascular Application,"* Pro. Int'l. Symp. Control. Rel. Bioact. Mater. 24:853-854; and Lam *et al.* (1997) *"Microencapsulation Of Recombinant Humanized Monoclonal Antibody For Local Delivery,"* Proc. Int'l. Symp. Control Rel. Bioact. Mater. 24:759-760, each of which is incorporated herein by reference in its entirety. In one embodiment, a pump may be used in a controlled-release system (*See* Langer, *supra*; Sefton, (1987) *"Implantable Pumps,"* CRC Crit. Rev. Biomed. Eng. 14:201-240; Buchwald *et al.* (1980) *"Long-Term, Continuous Intravenous Heparin Administration By An Implantable Infusion Pump In Ambulatory Patients With Recurrent Venous Thrombosis,"* Surgery 88:507-516; and Saudek *et al.* (1989) *"A Preliminary Trial Of The Programmable Implantable Medication System For Insulin Delivery,"* N. Engl. J. Med. 321:574-579). In another embodiment, polymeric materials can be used to achieve controlled-release of the molecules (*see e.g.*, MEDICAL APPLICATIONS OF CONTROLLED RELEASE, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); CONTROLLED DRUG BIOAVAILABILITY, DRUG PRODUCT DESIGN AND PERFORMANCE, Smolen and Ball (eds.), Wiley, New York (1984); Levy *et al.* (1985) *"Inhibition Of Calcification Of Bioprosthetic Heart Valves By Local Controlled-Release Diphosphonate,"* Science 228:190-192; During *et al.* (1989) *"Controlled Release Of Dopamine From A Polymeric Brain Implant: In Vivo Characterization,"* Ann. Neurol. 25:351-356; Howard *et al.* (1989) *"Intracerebral Drug Delivery In Rats With Lesion-Induced Memory Deficits,"* J. Neurosurg. 7(1):105-112); U.S. Patent No. 5,679,377; U.S. Patent No. 5,916,597; U.S. Patent No. 5,912,015; U.S. Patent No.

5,989,463; U.S. Patent No. 5,128,326; PCT Publication No. WO 99/15154; and PCT Publication No. WO 99/20253). Examples of polymers used in sustained-release formulations include, but are not limited to, poly(2-hydroxy ethyl methacrylate), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacrylic acid), polyglycolides (PLG), polyanhydrides, poly(N-vinyl pyrrolidone), poly(vinyl alcohol), polyacrylamide, poly(ethylene glycol), polylactides (PLA), poly(lactide-co-glycolides) (PLGA), and polyorthoesters. A controlled-release system can be placed in proximity of the therapeutic target (*e.g.*, the lungs), thus requiring only a fraction of the systemic dose (see, *e.g.*, Goodson, in MEDICAL APPLICATIONS OF CONTROLLED RELEASE, *supra*, vol. 2, pp. 115-138 (1984)). Polymeric compositions useful as controlled-release implants can be used according to Dunn *et al.* (See U.S. 5,945,155). This particular method is based upon the therapeutic effect of the in situ controlled-release of the bioactive material from the polymer system. The implantation can generally occur anywhere within the body of the patient in need of therapeutic treatment. A non-polymeric sustained delivery system can be used, whereby a non-polymeric implant in the body of the subject is used as a drug delivery system. Upon implantation in the body, the organic solvent of the implant will dissipate, disperse, or leach from the composition into surrounding tissue fluid, and the non-polymeric material will gradually coagulate or precipitate to form a solid, microporous matrix (See U.S. 5,888,533).

[00424] Controlled-release systems are discussed in the review by Langer (1990, *"New Methods Of Drug Delivery,"* Science 249:1527-1533). Any technique known to one of skill in the art can be used to produce sustained-release formulations comprising one or more therapeutic agents of the invention. See, *e.g.*, U.S. Patent No. 4,526,938; International Publication Nos. WO 91/05548 and WO 96/20698; Ning *et al.* (1996) *"Intratumoral Radioimmunotheraphy Of A Human Colon Cancer Xenograft Using A Sustained-Release Gel,"* Radiotherapy & Oncology 39:179-189, Song *et al.* (1995) *"Antibody Mediated Lung Targeting Of Long-Circulating Emulsions,"* PDA Journal of Pharmaceutical Science & Technology 50:372-397; Cleek *et al.* (1997) *"Biodegradable Polymeric Carriers For A bFGF Antibody For Cardiovascular Application,"* Pro. Int'l. Symp. Control. Rel. Bioact. Mater. 24:853-854; and Lam *et al.* (1997) *"Microencapsulation Of Recombinant Humanized Monoclonal Antibody For Local Delivery,"* Proc. Int'l. Symp. Control Rel. Bioact. Mater. 24:759-760, each of which is incorporated herein by reference in its entirety.

[00425] Where the composition of the invention is a nucleic acid encoding a PD-1-binding molecule of the present invention, the nucleic acid can be administered *in vivo* to promote expression of its encoded PD-1-binding molecule by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, *e.g.*, by use of a retroviral vector (*See* U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont), or coating with lipids or cell surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (See *e.g.*, Joliot *et al.* (1991) *"Antennapedia Homeobox Peptide Regulates Neural Morphogenesis,"* Proc. Natl. Acad. Sci. (U.S.A.) 88:1864-1868), *etc.* Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression by homologous recombination.

[00426] Treatment of a subject with a therapeutically or prophylactically effective amount of a PD-1-binding molecule of the present invention can include a single treatment or, preferably, can include a series of treatments. In a preferred example, a subject is treated with such a diabody one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. The pharmaceutical compositions of the invention can be administered once a day, twice a day, or three times a day. Alternatively, the pharmaceutical compositions can be administered once a week, twice a week, once every two weeks, once a month, once every six weeks, once every two months, twice a year or once per year. It will also be appreciated that the effective dosage of the molecules used for treatment may increase or decrease over the course of a particular treatment.

Examples

[00427] The following examples illustrate various methods for compositions in the diagnostic or treatment methods of the invention. The examples are intended to illustrate, but in no way limit, the scope of the invention.

Example 1
Characterization of Anti-Human PD-1 Monoclonal Antibodies

[00428] Fifteen murine monoclonal antibodies were isolated as being capable specifically binding to both human and cynomolgus monkey PD-1, and accorded the designations "**PD-1 mAb 1**," "**PD-1 mAb 2**," "**PD-1 mAb 3**," "**PD-1 mAb 4**," "**PD-1 mAb 5**," "**PD-1 mAb 6**," "**PD-1 mAb 7**," "**PD-1 mAb 8**," "**PD-1 mAb 9**," "**PD-1 mAb 10**," "**PD-1 mAb 11**," "**PD-1**

mAb 12," "**PD-1 mAb 13**," "**PD-1 mAb 14**," and "**PD-1 mAb 15**." The CDRs of these antibodies were found to differ and are provided above. Binding to the extracellular domain of human and cynomologus monkey PD-1 was evaluated as follows, flat bottom maxisorb 96-well plates were coated with soluble human or cynomolgus monkey PD-1 (the extracellular domain of human PD-1 fused to a His tag (shPD-1 His) or to a human Fc Region (shPD-1 hFc), or the extracellular domain of cynomolgus monkey PD-1 fused to a human Fc Region (scyno-PD1 Fc)) each at 0.5 or 1 μg/mL, the plates were washed and incubated with one of the isolated anti-PD-1 antibodies PD-1 mAb 1-15. For these studies the anti-PD-1 antibodies were utilized at 3, 1.0, 0.3333, 0.1111, 0.0370, 0.0123, or 0.0041 μg/mL (three fold serial dilutions). The amount of antibody binding to the immobilized PD-1 (human or cynomolgus monkey) was assessed using a goat anti-mouse IgG-HRP secondary antibody. All samples were analyzed on a plate reader (Victor 2 Wallac, Perkin Elmers). Representative binding curves for soluble human and soluble cynomolgus PD-1 are shown in **Figures 7A-7D** and **Figures 8A-8C**, respectively.

[00429] The results of these binding assays (**Figures 7A-7D** and **Figures 8A-8C**) show that all the anti-PD-1 antibodies PD-1 mAb 1-15 bind to both soluble human and soluble cynomolgus monkey PD-1.

[00430] In order to further characterize the murine anti-PD-1 antibodies their ability to block binding of soluble human PD-L1 to soluble human PD-1 was assessed in two different assays. In one assay the ability of the antibodies to block the binding of human PD-1 to PD-L1 immobilized on a surface was examined. For this assay each of the anti-PD-1 antibodies PD-1 mAb 1-15, or a reference anti-PD-1 antibody (PD-1 mAb A) was mixed with shPD-1 His fusion protein, (at 2.5 μg/mL) and was separately incubated with biotin labeled soluble human PD-L1 (the extracellular domain of PD-L1 fused to human Fc (sPD-L1)) at 1 μg/mL immobilized on a streptavidin coated plate. For these studies the anti-PD-1 antibodies were utilized at 10, 5.0, 2.5, 1.25, 0.625, 0.3125, or 0.1563 μg/mL (two fold serial dilutions). The amount of shPD-1 His binding to the immobilized sPD-L1 was assessed via the His-Tag using an anti-His-Tag-HRP secondary antibody. All samples were analyzed on a plate reader (Victor 2 Wallac, Perkin Elmers). The results of this experiment are shown in **Figures 9A-9D**.

[00431] The results of these inhibition assays (**Figures 9A-9D**) show that the anti-PD-antibodies PD-1 mAb 1, PD-1 mAb 3, PD-1 mAb 5, PD-1 mAb 6, PD-1 mAb 7, PD-1 mAb 8, PD-1 mAb 9, PD-1 mAb 10, PD-1 mAb 11, PD-1 mAb 12, PD-1 mAb 13, PD-1 mAb 14, and

PD-1 mAb 15, were able to block the binding of soluble human PD-L1 to soluble human PD-1 to varying degrees while PD-1 mAb 2 and PD-1 mAb 4 exhibited little to no blocking activity in this assay format.

[00432] In the second assay the ability of the murine anti-PD-1 antibodies PD-1 mAb 1-15 to block binding of PD-1 ligand (*i.e.*, human PD-L1 or human PD-L2) to PD-1 expressed on the surface of NSO cells was examined. For this assay each of the anti-PD-1 antibodies PD-1 mAb 1-15, or a reference anti-PD-1 antibody (PD-1 mAb A or PD-1 mAb B) was separately mixed with a biotinylated-soluble human PD-L1 (shPD-L1 fusion protein) or biotinylated-soluble human PD-L2-muIgFc fusion protein (shPD-L2; Ancell Cat# 573-030), each at 0.1 μg/test, and incubated with NSO cells expressing human PD-1 (~250,000 cells/well) in blocking buffer (FACS + 10% human serum albumin). For these studies the anti-PD-1 antibodies were utilized at 4.0, 1.0, 2.5 $x10^{-1}$, 6.25 x 10^{-2}, 1.56 x 10^{-2}, 3.90 x 10^{-3}, 9.76 x 10^{-4}, 2.4 x 10^{-4}, 0.6 x 10^{-4} μg/test (four fold serial dilutions). The amount of shPD-L1 (or shPD-L2) binding to the surface of the NSO cells was determined using a PE-conjugated Streptavidin secondary antibody by FACS analysis. The IC50 values for inhibition of PD-1/PD-L1 binding were determined and the sample mean (□) of at least two experiments are provided (except where indicated) in **Table 6**.

Table 6

Anti-PD-1 Antibody	IC50 (μg/test)		Anti-PD-1 Antibody	IC50 (μg/test)
PD-1 mAb A	0.0044		PD-1 mAb 8	0.6611 ‡
PD-1 mAb B	0.0064		PD-1 mAb 9	0.0154
PD-1 mAb 1	0.0048		PD-1 mAb 10	0.0057
PD-1 mAb 2	0.0110		PD-1 mAb 11	0.0259 ‡
PD-1 mAb 3	0.0361 ‡		PD-1 mAb 12	0.0238 ‡
PD-1 mAb 4	0.0156 ‡		PD-1 mAb 13	0.0117
PD-1 mAb 5	0.0039		PD-1 mAb 14	0.0149 ‡
PD-1 mAb 6	0.0051		PD-1 mAb 15	0.0060
PD-1 mAb 7	0.0024			

‡ Results from a single experiment

[00433] The results of the shPD-L1 inhibition assays (**Table 6**) show that the anti-PD-1 antibodies PD-1 mAb 1, PD-1 mAb 2, PD-1 mAb 3, PD-1 mAb 4, PD-1 mAb 5, PD-1 mAb 6, PD-1 mAb 7, PD-1 mAb 9, PD-1 mAb 10, PD-1 mAb 11, PD-1 mAb 12, PD-1 mAb 13, PD-1 mAb 14, and PD-1 mAb 15, were able to block the binding of human PD-L1 to human PD-1 expressed on the surface of NSO cells. In particular, PD-1 mAb 1, PD-1 mAb 5, PD-1 mAb 6, PD-1 mAb 7, PD-1 mAb 10, and PD-1 mAb 15 blocked shPD-L1 binding as well as or better than the reference PD-1 antibodies (PD-1 mAb A, PD-1 mAb B), while PD-1 mAb 8 was

essentially non-blocking in this assay format. Both PD-1 mAb 2 and PD-1 mAb 4 were able to block PD-1/PD-L1 binding in this assay format.

[00434] Similarly, the anti-PD-1 antibodies PD-1 mAb 1, PD-1 mAb 2, and PD-1 mAb 3, PD-1 mAb 4, PD-1 mAb 5, PD-1 mAb 6, PD-1 mAb 7, PD-1 mAb 9, PD-1 mAb 10, PD-1 mAb 12, PD-1 mAb 13, PD-1 mAb 14, were able to block the binding of human PD-L2 to human PD-1 expressed on the surface of NSO cells, while PD-1 mAb 8 was essentially non-blocking in this assay format. In particular, PD-1 mAb 1, PD-1 mAb 5, PD-1 mAb 6, PD-1 mAb 7, and PD-1 mAb 10 blocked shPD-L2 binding as well as, or better than the reference PD-1 antibodies (PD-1 mAb A, PD-1 mAb B). The PD-1 antibodies PD-1 mAb 11 and PD-1 mAb 15 were not tested in this assay. The results for several humanized anti-PD-1 antibodies including hPD-1 mAb 15 are provided below.

Example 2
Humanization and Further Characterization

[00435] The Variable Domains of the anti-PD-1 antibodies PD-1 mAb 1, PD-1 mAb 2, PD-1 mAb 7, PD-1 mAb 9, and PD-1 mAb 15 were humanized, where antigenic epitopes were identified the antibodies were further deimmunized to generate the final humanized Variable Domains. Humanization of PD-1 mAb 1, PD-1 mAb 2, and PD-1 mAb 15 yielded one humanized VH Domain and one humanized VL Domain for each antibody designated herein as **"hPD-1 mAb 1 VH1,"** and **"hPD-1 mAb 1 VL1**;" **"hPD-1 mAb 2 VH1,"** and **"hPD-1 mAb 2 VL1**;" and **"hPD-1 mAb 15 VH1,"** and **"hPD-1 mAb 15 VL1."** Humanization of PD-1 mAb 7 yielded two humanized VH Domains, designated herein as **"hPD-1 mAb 7 VH1,"** and **"hPD-1 mAb 7 VH2**," and three humanized VL Domains designated herein as **"hPD-1 mAb 1 VL1**," **"hPD-1 mAb 7 VL2**," and **"hPD-1 mAb 7 VL3**." Humanization of PD-1 mAb 9 yielded two humanized VH Domains, designated herein as **"hPD-1 mAb 9 VH1,"** and **"hPD-1 mAb 9 VH2**," and two humanized VL Domains designated herein as **"hPD-1 mAb 9 VL1**," and **"hPD-1 mAb 1 VL2**." Where multiple humanized Variable Domains were generated the humanized heavy and light chain Variable Domains of a particular anti-PD-1 antibody (*e.g.*, PD-1 mAb 7) may be used in any combination and particular combinations of humanized chains are referred to by reference to the specific VH/VL Domains, for example a humanized antibody comprising hPD-1 mAb 7 VH1 and hPD-1 mAb 7 VL2 is specifically referred to as **"hPD-1 mAb 7(1.2)**." Full length humanized antibodies were generated with either a human IgG1 constant region comprising the L234A/L235A substitutions (IgG1 (AA)) or a human IgG4 constant region comprising the S228P substitution (IgG4 (P)).

[00436] Full length IgG1 humanized antibody heavy chains were constructed as follows: the C-terminus of the humanized VH Domain was fused to the N-terminus of a human IgG1 Constant Region having a variant CH2-CH3 Domain (comprising the L234A/L235A (AA) substitutions) and lacking the C-terminal lysine residue (**SEQ ID NO:255**):

ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV
HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKRVEP
KSCDKTHTCP PCPAPE**AA**GG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS
HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK
EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSREE MTKNQVSLTC
LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW
QQGNVFSCSV MHEALHNHYT QKSLSLSPG

[00437] In **SEQ ID NO:255**, amino acid residues 1-98 correspond to the IgG1 CH1 Domain (**SEQ ID NO: 10**), amino acid residues 99-113 correspond to the IgG1 hinge region (**SEQ ID NO: 32**) and amino acid residues 114-329 correspond to the IgG1 CH2-CH3 Domain comprising the L234A/L235A substitutions (underlined) (**SEQ ID NO:5**) but lacking the C-terminal lysine residue.

[00438] The amino acid sequence of a heavy chain of an exemplary humanized antibody ((hPD-1 mAb 7(1.2)) having an IgG1 heavy chain constant region comprising the L234A/L235A mutation and lacking the C-terminal lysine residue is (**SEQ ID NO:265**):

QVQLVQSGAE VKKPGASVKV SCKASGYSFT SYWMNWVRQA PGQGLEWIGV
IHPSDSETWL DQKFKDRVTI TVDKSTSTAY MELSSLRSED TAVYYCAREH
YGTSPFAYWG QGTLVTVSSA STKGPSVFPL APSSKSTSGG TAALGCLVKD
YFPEPVTVSW NSGALTSGVH TFPAVLQSSG LYSLSSVVTV PSSSLGTQTY
ICNVNHKPSN TKVDKRVEPK SCDKTHTCPP CPAPE**AA**GGP SVFLFPPKPK
DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS
TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV
YTLPPSREEM TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL
DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPG

[00439] In **SEQ ID NO: 265**, amino acid residues 1-119 correspond to the VH Domain of hPD-1 mAb 7 VH1 (**SEQ ID NO: 147**), amino acid residues 120-217 correspond to the IgG1 CH1 Domain (**SEQ ID NO: 10**), residues 218-232 correspond to the IgG1 hinge region (**SEQ ID NO: 32**) and residues 233-448 correspond to the IgG1 CH2-CH3 Domain comprising the L234A/L235A substitutions (underlined) (**SEQ ID NO:5**) but lacking the C-terminal lysine residue.

[00440] Full length IgG4 humanized antibody heavy chains were constructed as follows: the C-terminus of the humanized VH Domain was fused to the N-terminus of a human IgG4 Constant Region having a stabilized hinge region (comprising the S228P substitution) and lacking the C-terminal lysine residue (**SEQ ID NO:256**):

ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV
HTFPAVLQSS GLYSLSSVVT VPSSSLGTKT YTCNVDHKPS NTKVDKRVES
KYGPPCP**P**CP APEFLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSQED
PEVQFNWYVD GVEVHNAKTK PREEQFNSTY RVVSVLTVLH QDWLNGKEYK
CKVSNKGLPS SIEKTISKAK GQPREPQVYT LPPSQEEMTK NQVSLTCLVK
GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSRL TVDKSRWQEG
NVFSCSVMHE ALHNHYTQKS LSLSLG

[00441] In **SEQ ID NO:256**, amino acid residues 1-98 correspond to the IgG4 CH1 Domain (**SEQ ID NO:254**), amino acid residues 99-110 correspond to the stabilized IgG4 hinge region comprising the S228P substitutions (underlined) (**SEQ ID NO: 13**) and amino acid residues 111-326 correspond to the IgG4 CH2-CH3 Domain (**SEQ ID NO:4**) but lacking the C-terminal lysine residue.

[00442] The amino acid sequence of a heavy chain of an exemplary humanized antibody ((hPD-1 mAb 7(1.2)) having an IgG4 heavy chain constant region comprising a stabilized hinge region having the S228P mutation and lacking the C-terminal lysine residue is (**SEQ ID NO:266**):

QVQLVQSGAE VKKPGASVKV SCKASGYSFT SYWMNWVRQA PGQGLEWIGV
IHPSDSETWL DQKFKDRVTI TVDKSTSTAY MELSSLRSED TAVYYCAREH
YGTSPFAYWG QGTLVTVSSA STKGPSVFPL APCSRSTSES TAALGCLVKD
YFPEPVTVSW NSGALTSGVH TFPAVLQSSG LYSLSSVVTV PSSSLGTKTY
TCNVDHKPSN TKVDKRVESK YGPPCP**P**CPA PEFLGGPSVF LFPPKPKDTL
MISRTPEVTC VVVDVSQEDP EVQFNWYVDG VEVHNAKTKP REEQFNSTYR
VVSVLTVLHQ DWLNGKEYKC KVSNKGLPSS IEKTISKAKG QPREPQVYTL
PPSQEEMTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTPPVLDSD
GSFFLYSRLT VDKSRWQEGN VFSCSVMHEA LHNHYTQKSL SLSLG

[00443] In **SEQ ID NO:266**, amino acid residues 1-119 correspond to the VH Domain of hPD-1 mAb 7 VH1 (**SEQ ID NO:147**), amino acid residues 120-217 correspond to the IgG4 CH1 Domain (**SEQ ID NO:254**), amino acid residues 218-229 correspond to the stabilized IgG4 hinge region comprising the S228P substitutions (underlined) (**SEQ ID NO:13**) and amino acid residues 230-445 correspond to the IgG4 CH2-CH3 Domain (**SEQ ID NO:4**) but lacking the C-terminal lysine residue.

[00444] Full length humanized antibody light chains were constructed as follows: the C-terminus of the humanized VL Domain was fused to the N-terminus of a human light chain kappa region (**SEQ ID NO:8**). The same light chain is paired with the IgG1 (AA) and the IgG4 (P) heavy chains.

[00445] The amino acid sequence of a light chain of an exemplary humanized PD-1 antibody (hPD-1 mAb 7(1.2)) having a kappa constant region is (**SEQ ID NO:264**):

EIVLTQSPAT LSLSPGERAT LSCRASESVD NYGMSFMNWF QQKPGQPPKL
LIHAASNQGS GVPSRFSGSG SGTDFTLTIS SLEPEDFAVY FCQQSKEVPY
TFGGGTKVEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV
QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV
THQGLSSPVT KSFNRGEC

[00446] In **SEQ ID NO:264**, amino acid residues 1-111 correspond to the VL Domain of hPD-1 mAb 7 VL2 (**SEQ ID NO:151**), and amino acid residues 112-218 correspond to the light chain kappa constant region (**SEQ ID NO: 8**).

[00447] Anti-PD-1 antibodies having alternative Constant Regions, for example Engineered Fc Regions, are readily generated by incorporating different Constant Regions and/or by introducing one or more amino acid substitutions, additions or deletions. For example, where a bispecific antibody is desired knob-bearing and hole-bearing CH2-CH3 domains are used to facilitate heterodimerization. Chimeric anti-PD-1 antibodies comprising the murine Variable Domains and human Constant Regions are generated as described above.

[00448] The humanized antibodies (IgG1 (AA) and/or IgG4 (P)) were tested for binding and blocking activity as described in above. The binding to human PD-1 (shPD-1 His, and shPD-1 hFc) and cynomolgus monkey PD-1 (shPD-L1 hFc) of the humanized antibodies was comparable to that of the corresponding murine antibody. In addition, the humanized antibodies retained the ability to block the binding of human PD-L1 to human PD-1 in an ELISA assay.

[00449] The binding kinetics of the murine antibodies PD-1 mAb 2, PD-1 mAb 7, PD-1 mAb 9, PD-1 mAb 15, the humanized antibodies hPD-1 mAb 2, hPD-1 mAb 7(1.2), hPD-1 mAb 9(1.1), hPD-1 mAb 15, and the reference anti-PD-1 antibodies PD-1 mAb A and PD-1 mAb B was investigated using Biacore analysis. The anti-PD-1 antibodies were captured on immobilized Protein A and were incubated with His-tagged soluble human PD-1 (shPD-1-His) or soluble human cynomolgus monkey PD-1 Fc fusion (scyno PD-1 hFc) cleaved to remove

Fc portion, and the kinetics of binding was determined via Biacore analysis. In additional studies the anti-PD-1 antibodies hPD-1 mAb 7(1.2) IgG1 (AA), hPD-1 mAb 7(1.2) IgG4 (P), hPD-1 mAb 9(1.1) IgG1 (AA), hPD-1 mAb 9(1.1) IgG4 (P), PD-1 mAb A IgG1 (AA), PD-1 mAb A IgG4 (P), PD-1 mAb B IgG1 (AA), and PD-1 mAb B IgG4 (P), were captured on immobilized $F(ab)_2$ goat anti-human Fc and the binding kinetics were determined by Biacore analysis as described above. The calculated ka, kd and KD from these studies are presented in **Table 7**.

Table 7

Protein A Capture							
Anti-PD-1 Antibody	**Human[a]**				**Cynomolgus Monkey[b]**		
	k_a ($x10^4$)	**k_d ($x10^{-4}$)**	**KD (nM)**		**k_a ($x10^4$)**	**k_d ($x10^{-4}$)**	**KD (nM)**
PD-1 mAb A	60	18	3		14	9.6	6.9
PD-1 mAb B	140	35	2.5		37	12	3.2
PD-1 mAb 7	21	2.8	1.3		17	6	3.5
hPD-1 mAb 7(1.2)	110	4.3	0.39		37	6.4	1.7
PD-1 mAb 9	4.3	4.2	9.8		2.2	16	72.7
hPD-1 mAb 9(1.1)	1.8	6.5	36.1		1.5	11	73.3
PD-1 mAb 15	4.5	1.3	2.9		2.7	11	40.7
hPD-1 mAb 15	2.4	3.2	13.3		2.3	18	78.3
PD-1 mAb 2	5.5	5.6	10.2		4.2	6.0	14.3
hPD-1 mAb 2	3.2	1.6	5.0		2.3	3.9	17
$F(ab)_2$ goat anti-human Fc Capture							
PD-1 mAb A IgG1 (AA)	13	8.4	6.5		8.1	4.5	5.6
PD-1 mAb A IgG4 (P)	13	7.9	6.1		8.4	5.0	6.0
PD-1 mAb B IgG1 (AA)	25	28	11.2		20	6.4	3.2
PD-1 mAb B IgG4 (P)	26	25	9.6		20	7.9	4.0
hPD-1 mAb 7(1.2) IgG1 (AA)	25	3.8	1.5		16	7.8	4.9
hPD-1 mAb 7(1.2) IgG4 (P)	27	4.1	1.5		17	7.8	4.6
hPD-1 mAb 9(1.1) IgG1 (AA)	5.6	6.1	10.9		5.6	5.2	9.3
hPD-1 mAb 9(1.1) IgG4 (P)	6.1	5.8	9.5		4.9	7.4	15.1

[a]His tagged soluble human PD-1 (shPD-1 His)
[b]soluble cynomolgus monkey PD-1 (scyno PD-1 hFc) cleaved

[00450] The results demonstrate that PD-1 mAb 7 and the humanized hPD-1 mAb 7(1.2) exhibit better binding kinetics relative to the reference anti-PD-1 antibodies PD-1 mAb A and PD-1 mAb B. PD-1 mAb 2, and hPD-1 mAb 2 exhibit binding kinetics within about two fold of the reference anti-PD-1 antibodies while PD-1 mAb 9, hPD-1 mAb 9(1.1), PD-1 mAb 15, and hPD-1 mAb 15 exhibit binding kinetics within about 2-6 fold of the reference anti-PD-1 antibodies.

[00451] The tissue specificity of the anti-human PD-1 antibody PD-1 mAb 7 was investigated. Normal tissue was contacted with PD-1 mAb 7 or with an isotype control (0.313 μg/mL) and the extent of staining was visualized. Bloxall used for endogenous enzyme block to reduce non-specific mucin staining in the colon tissue. As shown in **Figure 10A, Panels i-xii**, PD-1 mAb 7 and the isotype control both failed to label cells of normal colon, liver, lung, pancreas, kidney and heart tissue. In addition, PD-1 mAb 7 and the isotype control failed to stain normal skin (**Figure 10B, Panels i-ii**). In contrast, PD-1 mAb 7 was found to strongly label lymphocytes present in normal tonsil tissue and PDCD1 transfected NSO cells expressing PD-1 (**Figure 10B, Panels iii and v**), while the isotype control failed to label either (**Figure 10B, Panels iv and vi)**. The results presented in **Figures 10A-10B** thus indicate that PD-1 mAb 7 was capable of specifically binding to lymphocytes and cells expressing PD-1.

[00452] The binding saturation profiles of hPD-1 mAb 2 IgG1 (AA), hPD-1 mAb 7(1.1) IgG1 (AA), hPD-1 mAb 7(1.2) IgG1, (AA), hPD-1 mAb 7(1.2) IgG4 (P), hPD-1 mAb 9(1.1) IgG1 (AA), hPD-1 mAb 9(1.1) IgG4 (P), hPD-1 mAb 15 IgG1 (AA), and the reference anti-PD-1 antibodies PD-1 mAb A and PD-1 mAb B was examined. Briefly, each of the anti-PD-1 antibodies, PD-1 mAb 1-15, or the reference anti-PD-1 antibodies (PD-1 mAb A and PD-1 mAb B) was mixed with NSO cells expressing human PD-1 (~250,000 cells/well) in blocking buffer (FACS + 10% human serum albumin). For these studies the anti-PD-1 antibodies were utilized at 50, 12.5, 3.13, 2.0 $x10^{-1}$, 4.9 x 10^{-2}, 1.2 x 10^{-2}, 3.0 x 10^{-3}, 1.9 x 10^{-4}, 7.6 x 10^{-4}, 4.75 x 10^{-5}, or 1.19 x 10^{-5} μg/test (four fold serial dilutions). The amount of antibody binding to the surface of the NSO cells was determined using goat anti-human-APC secondary antibody by FACS analysis. Representative saturation curves are shown in **Figure 11**. The EC50 and EC90 values were determined and the sample mean (SM) and standard deviation (SD **σ**) from four separate experiments are provided in **Table 8**.

Table 8				
	Saturation Binding			
	EC50 (μg/test)		EC90 (μg/test)	
Anti-PD-1 Antibody	SM	SD σ	SM	SD σ
PD-1 mAb A IgG1 (AA)	0.1991	0.1309	1.4528	0.8040
PD-1 mAb A IgG4 (P)	0.1581	0.1161	1.5464	1.7690
PD-1 mAb B IgG1 (AA)	0.1347	0.0681	1.3917	0.9573
PD-1 mAb B IgG4 (P)	0.1398	0.0951	1.1619	1.2681
hPD-1 mAb 2 IgG1 (AA)	0.4431	0.1997	2.4374	1.2637
hPD-1 mAb 7(1.1) IgG1 (AA)	0.1069	0.0500	0.9102	0.5476
hPD-1 mAb 7(1.2) IgG1 (AA)	0.1872	0.1553	0.6810	0.3226
hPD-1 mAb 7(1.2) IgG4 (P)	0.1376	0.0926	0.6609	0.3437
hPD-1 mAb 9(1.1) IgG1 (AA)	0.3123	0.2291	1.6486	0.9117
hPD-1 mAb 9(1.1) IgG4 (P)	0.5128	0.2228	3.0563	0.9437
hPD-1 mAb 15 IgG1 (AA)	0.2927	0.1333	2.0640	0.6096

[00453] The binding saturation studies demonstrate that the humanized versions of PD-1 mAb 2, PD-1 mAb 7, PD-1 mAb 9, and PD-1 mAb 15 have favorable profile for binding to cell surface PD-1. In particular, humanized PD-1 mAb 7 (hPD-1 mAb 7(1.1), and hPD-1 mAb 7(1.2) having either an IgG1 (AA) or an IgG4 (P) Fc Region) have the lowest EC90 values of all the antibodies examined.

[00454] In order to further characterize the humanized anti-PD-1 antibodies hPD-1 mAb 2 IgG1 (AA), hPD-1 mAb 7(1.1) IgG1 (AA), hPD-1 mAb 7(1.2) IgG1, (AA), hPD-1 mAb 7(1.2) IgG4 (P), hPD-1 mAb 9(1.1) IgG1 (AA), hPD-1 mAb 9(1.1) IgG4 (P), and hPD-1 mAb 15 IgG1 (AA), their ability to block binding of human PD-L1 (shPD-L1) and human PD-L2 (shPD-L2) to PD-1 expressed on the surface of NSO cells was examined. These assays were performed essentially as described above. Representative curves for inhibition of sPD-L1 and sPD-L2 binding to PD-1 expressed on NSO cells are shown in **Figures 12A** and **12B**, respectively. The IC50 and IC90 values were determined and the sample mean (SM) and standard deviation (SD **σ**) from three separate experiments are provided in **Table 9**.

Table 9								
	sPD-L1				sPD-L2			
	IC50 (μg/test)		IC90 (μg/test)		IC50 (μg/test)		IC90 (μg/test)	
Anti-PD-1 Antibody	SM	SD σ	SM	SD σ	SM	SD σ	SM	SD σ
PD-1 mAb A IgG1 (AA)	0.0203	0.0089	0.2985	0.3279	0.0414	0.0124	0.1601	0.066
PD-1 mAb A IgG4 (P)	0.0156	0.0096	0.0776	0.0208	0.0280	0.0070	0.1594	0.1153
PD-1 mAb B IgG1 (AA)	0.0148	0.0008	0.1034	0.0100	0.0280	0.0059	0.1190	0.060
PD-1 mAb B IgG4 (P)	0.0143	0.0013	0.0798	0.0239	0.0280	0.0055	0.0924	0.0065
hPD-1 mAb 2 IgG1 (AA)	0.0578	0.0124	0.2480	0.050	0.1294	0.0143	0.3813	0.0656
hPD-1 mAb 7(1.1) IgG1 (AA)	0.0166	0.0032	0.0674	0.0041	0.0283	0.0147	0.0886	0.0166
hPD-1 mAb 7(1.2) IgG1 (AA)	0.0118	0.0027	0.0678	0.0031	0.0212	0.0031	0.0672	0.0043
hPD-1 mAb 7(1.2) IgG4 (P)	0.0103	0.0023	0.0520	0.0033	0.0213	0.0019	0.0616	0.0063
hPD-1 mAb 9(1.1) IgG1 (AA)	0.0593	0.0036	0.3238	0.0508	0.4002	0.5000	0.4573	0.1805
hPD-1 mAb 9(1.1) IgG4 (P)	0.0460	0.0118	0.2461	0.0513	0.1105	0.0146	0.2914	0.0526
hPD-1 mAb 15 IgG1 (AA)	0.0440	0.0092	0.2068	0.035	0.0945	0.0022	0.3093	0.0588

[00455] The ligand binding inhibition studies demonstrate that the humanized versions of PD-1 mAb 2, PD-1 mAb 7, PD-1 mAb 9, and PD-1 mAb 15 are able to inhibit the binding of sPD-L1 and sPD-L2 to PD-1 on the cell surface. In particular, humanized PD-1 mAb 7 (hPD-1 mAb 7(1.1), and hPD-1 mAb 7(1.2)) have the lowest IC90 values of all the antibodies examined.

Example 3
Blockade of the PD-1/PD-L1 Checkpoint by Humanized Anti-Human PD-1 Antibodies

[00456] The ability of hPD-1 mAb 2 IgG1 (AA), hPD-1 mAb 7(1.1) IgG1 (AA), hPD-1 mAb 7(1.2) IgG1, (AA), hPD-1 mAb 7(1.2) IgG4 (P), hPD-1 mAb 9(1.1) IgG1 (AA), hPD-1 mAb 9(1.1) IgG4 (P), hPD-1 mAb 15 IgG1 (AA), and the reference anti-PD-1 antibodies PD-1 mAb A and PD-1 mAb B to antagonize the PD-1/PD-L1 axis (*i.e.*, block the PD-1/PD-L1 interaction and prevent down-regulation of T-cell responses) was examined in a Jurkat-luc-NFAT / CHO-PD-L1 luciferase reporter assay. Briefly, CHO cells expressing PD-L1 (CHO/PD-L1) were plated at 40,000/well in 100 μL of culture medium (RPMI + 10% FBS + 100 μg/mL

Hygromycine B + 100 μg/mL G418) and incubated overnight. The next day the media was removed and MNFAT-luc2/PD-1 Jurkat cells (Promega) at 50,000 cells/well in 40 μL in assay buffer (RPMI + 2% FBS), and the anti-PD-1 antibodies PD-1 mAb 1-15, or a reference anti-PD-1 antibodies (PD-1 mAb A and PD-1 mAb B) (0-25 μg/mL; eight 2.5 fold serial dilutions in assay buffer) were added to each well and incubated for 6 hours at 37°C followed by a 5-10 minutes incubated at ambient temperature. 80 μL of BioGlo Substrate (Promega) was then added to each well and the plate was incubated for an additional 5-10 minutes at ambient temperature, the luminescence was measured in a Victor Plate Reader. Representative saturation curves are shown in **Figure 13**. The EC50 and EC90 values were determined and the sample mean (SM) and standard deviation (SD **σ**) from four separate experiments are provided in **Table 10**.

Table 10

Anti-PD-1 Antibody	Reporter Signaling			
	EC50 (μg/test)		EC90 (μg/test)	
	SM	SD σ	SM	SD σ
PD-1 mAb A IgG1 (AA)	0.2549	0.0480	2.4474	1.2228
PD-1 mAb A IgG4 (P)	0.2049	0.0719	2.5535	1.2139
PD-1 mAb B IgG1 (AA)	0.2119	0.1781	2.2036	2.0118
PD-1 mAb B IgG4 (P)	0.1142	0.0323	0.9418	0.2863
hPD-1 mAb 2 IgG1 (AA)	0.3539	0.0983	3.8975	2.0054
hPD-1 mAb 7(1.1) IgG1 (AA)	0.1080	0.0386	1.1992	0.5103
hPD-1 mAb 7(1.2) IgG1 (AA)	0.0944	0.0153	0.6452	0.2615
hPD-1 mAb 7(1.2) IgG4 (P)	0.0965	0.0169	0.6885	.01858
hPD-1 mAb 9 IgG1 (AA)	0.2835	0.0530	2.9968	0.8866
hPD-1 mAb 9 IgG4 (P)	0.3154	0.0872	5.0940	4.0496
hPD-1 mAb 15 IgG1 (AA)	0.2585	0.0592	3.3138	1.0532

[00457] The reporter signaling studies demonstrate that the humanized versions of PD-1 mAb 2, PD-1 mAb 7, PD-1 mAb 9, and PD-1 mAb 15 can block the PD-1/PD-L1 axis and will prevent down-regulation of T-cell responses. In particular, humanized PD-1 mAb 7 (hPD-1 mAb 7(1.1), and hPD-1 mAb 7(1.2) having either an IgG1 (AA) or an IgG4 (P) Fc Region) have the lowest EC50/EC90 values.

Example 4
Functional Activity of Anti-Human PD-1 Antibodies

[00458] *Staphylococcus aureus* enterotoxin type B (SEB) is a microbial superantigen capable of activating a large proportion of T-cells (5-30%) in SEB-responsive donors. SEB binds to MHC II outside the peptide binding grove and thus is MHC II dependent, but unrestricted and TCR mediated. SEB-stimulation of T-cells results in oligoclonal T-cell proliferation and cytokine production (although donor variability may be observed and some donors will not respond). Within 48 hours of SEB-stimulation PMBCs upregulate PD-1 and LAG-3 with a further enhancement see at day 5, post-secondary culture in 96-well plate with SEB-stimulation. Upregulation of the immune check point proteins PD-1 and LAG-3 following SEB-stimulation of PBMCs limits cytokine release upon restimulation. The ability of anti-PD-1 antibodies alone and in combination with anti-LAG-3 antibodies to enhance cytokine release through checkpoint inhibition was examined.

[00459] Briefly, PBMCs were purified using the Ficoll-Paque Plus (GE Healthcare) density gradient centrifugation method according to manufacturer's instructions from whole blood obtained under informed consent from healthy donors (Biological Specialty Corporation) and T cells were then purified using the Dynabeads® Untouched Human T Cells Kit (Life Technologies) according to manufacturer's instructions. Purified PBMCs were cultured in RPMI-media + 10% heat inactivated FBS + 1% Penicillin/Streptomycin in T-25 bulk flasks for 2-3 days alone or with SEB (Sigma-Aldrich) at 0.1 ng/mL (primary stimulation). At the end of the first round of SEB-stimulation, PBMCs were washed twice with PBS and immediately plated in 96-well tissue culture plates at a concentration of 1-5 x 10^5 cells/well in media alone, media with a control or an anti-PD-1 antibody, media with SEB at 0.1 ng/mL (secondary stimulation) and no antibody, or media with SEB and a control IgG or an anti-PD-1 antibody +/- an anti-LAG-3 mAb, and cultured for an additional 2-3 days. At the end of the second stimulation, supernatants were harvested to measure cytokine secretion using human DuoSet ELISA Kits for IFNγ, TNFα, IL-10, and IL-4 (R&D Systems) according to the manufacturer's instructions.

[00460] The ability of PD-1 mAb 2, PD-1 mAb 7, PD-1 mAb 9, and PD-1 mAb 15 alone, or in combination with the unique anti-LAG-3 antibody LAG-3 mAb 1 to enhance cytokine release through checkpoint inhibition was examined. These studies also included one or more of the following reference anti-PD-1 antibodies: PD-1 mAb A; PD-1 mAb B; and LAG-3 mAb

A, alone or in combination. **Figure 14** shows the IFNγ secretion profiles from SEB-stimulated (0.1 ng/mL) PBMCs from a representative responding donor (D:38941), treated with: no antibody; isotype control antibody; PD-1 mAb 7 and/or LAG-3 mAb 7; PD-1 mAb 9 and/or LAG-3 mAb 1; PD-1 mAb 15 and/or LAG-3 mAb 1; PD-1 mAb 2 and/or LAG-3 mAb 1; or the reference anti-PD-1 antibodies PD-1 mAb B and/or LAG-3 mAb A (antibodies were used at 10 μg/mL).

[00461] In additional studies the ability of the humanized versions of PD-1 mAb 2, PD-1 mAb 7, PD-1 mAb 9, and PD-1 mAb 15 (comprising a human IgG1 (AA) or a human IgG4 (P) Fc Region) as well as the reference anti-PD-1 antibodies PD-1 mAb A and PD-1 mAb B to enhance cytokine release through checkpoint inhibition was examined. For these studies the antibodies were utilized at 0.625, 2.5, and 10 μg/mL. **Figures 15A-15B** shows the IFNγ (**Figure 15A**) and TNFα (**Figure 15B**), secretion profiles from SEB-stimulated (0.2 ng/mL) PBMCs from a representative responding donor (D:57709), treated with no antibody or one of the following antibodies: isotype control; hPD-1 mAb 2 IgG1 (AA); hPD-1 mAb 7(1.2) IgG1 (AA); hPD-1 mAb 7(1.2) IgG4 (P); hPD-1 mAb 9(1.1) IgG1 (AA); hPD-1 mAb 9(1.1) IgG4 (P); hPD-1 mAb 15 IgG1 (AA); or the reference anti-PD-1 antibodies PD-1 mAb A IgG1 (AA), PD-1 mAb A IgG4 (P), PD-1 mAb B IgG1 (AA), PD-1 mAb B IgG4 (P). The total pg/mg of IFNγ in samples treated with SEB+Ab were determined for the samples treated with the anti-PD-1 antibodies at 0.625, 2.5 and 10 μg/mL and the sample mean (SM) and standard deviation (SD **σ**) from 3 different responding donors (except where noted) are provided in **Table 11**. The ratio of IFNγ secreted in sample treated with the humanized versions of PD-1 mAb 2, PD-1 mAb 7, PD-1 mAb 9, and PD-1 mAb 15 (comprising a human IgG1 (AA) or a human IgG4 (P) Fc Region) over the reference anti-PD-1 antibodies PD-1 mAb A and PD-1 mAb B (*i.e.*, humanized anti-PD-1/PD-1 mAb A, and humanized anti-PD-1/PD-1 mAb B) is presented in **Table 12** and **Table 13**, respectively.

Table 11						
	IFNγ Secretion (pg/mL)					
µg/mL anti-PD1 antibody	0.625 µg/mL		2.5 µg/mL		10 µg/mL	
anti-PD-1 Antibody	**SM**	**SD σ**	**SM**	**SD σ**	**SM**	**SD σ**
PD-1 mAb A IgG1 (AA)	221.18	110.89	341.13	247.93	347.46	144.72
PD-1 mAb A IgG4 (P)	281.36	132.65	495.15	190.57	399.41	117.56
PD-1 mAb B IgG1 (AA)	366.69	196.64	387.682	215.51	387.32	282.81
PD-1 mAb B IgG4 (P)	348.40	185.96	433.382	163.23	551.68	125.08
hPD-1 mAb 7(1.2) IgG1 (AA)	302.05	185.71	610.70	209.77	414.63	272.65
hPD-1 mAb 7(1.2) IgG4 (P)	384.57‡	323.79‡	411.40	398.59	370.06	108.12
hPD-1 mAb 9(1.1) IgG1 (AA)	340.81	207.76	442.598	303.70	655.29	567.91
hPD-1 mAb 9(1.1) IgG4 (P)	309.82	130.30	468.62	350.15	424.35	288.95
hPD-1 mAb 15 IgG1 (AA)	360.00	274.28	373.32	160.25	541.83	444.22
hPD-1 mAb 2 IgG1 (AA)	275.88	135.23	372.73	53.53	496.70	235.37
Control IgG	137.14	76.61	100.65	48.67	138.10	120.81
No Antibody	120.05	73.90	120.05	73.90	109.46	85.18

‡ Results from two responding donors

Table 12						
	Ratio IFNγ Secretion (New Anti-PD-1/PD-1 mAb A)					
µg/mL anti-PD1 antibody	0.625 µg/mL		2.5 µg/mL		10 µg/mL	
anti-PD-1 Antibody	**SM**	**SD σ**	**SM**	**SD σ**	**SM**	**SD σ**
PD-1 mAb A IgG1 (AA)	1.00	0.00	1.00	0.00	1.00	0.00
PD-1 mAb A IgG4 (P)	1.00	0.00	1.00	0.00	1.00	0.00
PD-1 mAb B IgG1 (AA)	1.77	0.92	1.28	0.36	1.07	0.42
PD-1 mAb B IgG4 (P)	1.23	0.16	0.92	0.27	1.40	0.12
hPD-1 mAb 7(1.2) IgG1 (AA)	1.36	0.37	2.46	1.85	1.17	0.41
hPD-1 mAb 7(1.2) IgG4 (P)	1.20‡	0.35‡	0.79	0.54	0.95	0.22
hPD-1 mAb 9(1.1) IgG1 (AA)	1.48	0.19	1.46	0.71	1.70	0.84
hPD-1 mAb 9(1.1) IgG4 (P)	1.13	0.13	0.91	0.42	1.02	0.46
hPD-1 mAb 15 IgG1 (AA)	1.50	0.39	1.51	1.23	1.48	0.71
hPD-1 mAb 2 IgG1 (AA)	1.32	0.53	1.48	0.86	1.42	0.12
Control IgG	0.63	0.2	0.33	0.08	0.39	0.24
No Antibody	0.54	0.12	0.39	0.14	0.31	0.17

‡ Results from two responding donors

Table 13						
	Ratio IFNγ Secretion (New Anti-PD-1/PD-1 mAb B)					
μg/mL anti-PD1 antibody	0.625 μg/mL		2.5 μg/mL		10 μg/mL	
anti-PD-1 Antibody	**SM**	**SD σ**	**SM**	**SD σ**	**SM**	**SD σ**
PD-1 mAb A IgG1 (AA)	0.37	0.37	0.82	0.20	1.06	0.48
PD-1 mAb A IgG4 (P)	0.82	0.12	1.16	0.38	0.72	0.07
PD-1 mAb B IgG1 (AA)	1.0	0.00	1.0	0.00	1.0	0.00
PD-1 mAb B IgG4 (P)	1.0	0.00	1.0	0.00	1.0	0.00
hPD-1 mAb 7(1.2) IgG1 (AA)	0.84	0.22	1.77	0.81	1.11	0.07
hPD-1 mAb 7(1.2) IgG4 (P)	0.91‡	0.26‡	0.83	0.50	0.68	0.17
hPD-1 mAb 9(1.1) IgG1 (AA)	1.04	0.59	1.12	0.29	1.60	0.42
hPD-1 mAb 9(1.1) IgG4 (P)	0.92	0.09	0.99	0.36	0.75	0.39
hPD-1 mAb 15 IgG1 (AA)	1.01	0.48	1.07	0.57	1.34	0.15
hPD-1 mAb 2 IgG1 (AA)	0.78	0.12	1.10	0.38	1.46	0.53
Control IgG	0.39	0.08	0.27	0.08	0.34	0.13
No Antibody	0.34	0.11	0.31	0.03	0.28	0.08

‡ Results from two responding donors

[00462] The results of these studies demonstrate that the PD-1 antibodies PD-1 mAb 2, PD-1 mAb 7, PD-1 mAb 9, and PD-1 mAb 15 dramatically enhanced IFNγ (**Figures 14** and **15A**, and **Tables 11-13**), and TNFα (**Figure 15B**) production from SEB-stimulated PBMCs upon restimulation. In addition, the combination of anti-PD-1 antibodies with anti-LAG-3 antibodies resulted in a further enhancement of cytokine release (**Figure 14**) from SEB-stimulated PBMCs upon restimulation. In particular, the combination of PD-1 mAb 2, PD-1 mAb 7, PD-1 mAb 9, or PD-1 mAb 15 with the unique anti-LAG-3 antibody LAG-3 mAb 1 provided the largest enhancement.

Example 5
PD-1 x LAG-3 Bispecific Molecules Binding Studies

[00463] A number of PD-1 x LAG-3 bispecific molecules were generated, including Fc Region-containing diabodies comprising three, four, and five chains and a bispecific antibody. Four diabodies having four chains and comprising E/K-coil Heterodimer-Promoting Domains were generated and accorded the designations "**DART A**," "**DART B**," "**DART C**, and "**DART I**." Four diabodies having four chains and comprising CH1/CL Domains were generated and accorded the designations "**DART D**," "**DART E**," "**DART J**," and "**DART 1**." Two diabodies having five chains and comprising E/K-coil Heterodimer-Promoting Domains and CH1/CL Domains were generated and accorded the designations "**DART F**," and "**DART G**." One diabody having three chains and comprising E/K-coil Heterodimer-Promoting Domains was generated and accorded the designation "**DART H**." One bispecific antibody having four chains was generated and accorded the designation "**BSAB A**." The

structure and amino acid sequences of these PD-1 x LAG-3 bispecific molecules are provided above and are summarized in **Table 14** below.

Table 14

Name	Parental mAbs	Fc‡	Chains	SEQ ID NOs:	Other Components
DART A	hPD-1 mAb 7(1.2) hLAG-3 mAb 1(1.4)	IgG4 (YTE)	4	267 (X_1=A; X_2=Y; X_3=T; X_4=E) and 268	E/K-Coils; see Figure 3B
DART B	hPD-1 mAb 7(1.2) hLAG-3 mAb 1(1.3)	IgG4 (YTE)	4	267 (X_1=G; X_2=Y; X_3=T; X_4=E) and 268	E/K-Coils; see Figure 3B
DART C	hPD-1 mAb 7(1.2) hLAG-3 mAb 1(1.3)	IgG4	4	267 (X_1=G; X_2=M; X_3=S; X_4=T) and 268	E/K-Coils; see Figure 3B
DART D	hPD-1 mAb 7(1.2) hLAG-3 mAb 1(1.4)	IgG4 (YTE)	4	269 and 270	CL/CH1; see Figure 3C
DART E	hPD-1 mAb 7(1.2) hLAG-3 mAb 1(1.4)	IgG4 (YTE)	4	271 and 272	CL/CH1; see Figure 3C
DART F	hPD-1 mAb 7(1.2) hLAG-3 mAb 1(1.4)	IgG1 (AA/ YTE)	5	273, 274, 275, and 276	CL/CH1 and E/K-Coils; see Figure 5
DART G	hPD-1 mAb 7(1.2) hLAG-3 mAb 1(1.4)	IgG1 (AA/ YTE)	5	277, 278, 279, and 280	CL/CH1 and E/K-Coils; see Figure 5
DART H	hPD-1 mAb 7(1.2) hLAG-3 mAb 1(1.4)	IgG1 (AA)	3	281, 282, and 283	E/K Coils; See Figure 4A
DART I	hPD-1 mAb 7(1.2) hLAG-3 mAb 6(1.1)	IgG4 (YTE)	4	290 and 291	E/K-Coils; see Figure 3B
DART J	hPD-1 mAb 7(1.2) hLAG-3 mAb 6(1.1)	IgG4 (YTE)	4	292 and 293	CL/CH1; see Figure 3C
DART 1	PD-1 mAb A LAG-3 mAb A	IgG1 (AA)	4	284 and 285	CL/CH1; see Figure 3C
BSAB A	hPD-1 mAb 7(1.2) hLAG-3 mAb 1(1.4)	IgG1 (AA)	4	286, 287, 288, and 289	mAb with charge engineered Fc Region

‡ Molecules incorporating IgG4 Fc regions also incorporate a stabilized IgG4 hinge region.

[00464] Additional PD-1 x LAG-3 bispecific molecules comprising alternative PD-1 and/or LAG-3 epitope-binding sites may be readily generated by incorporating different VH and VL Domains. Similarly, molecules binding an antigen other than LAG-3 may be generated by incorporating the VH and VL having the desired specificity.

[00465] The binding saturation profiles of the PD-1 x LAG-3 bispecific diabody constructs: DART A, DART B, DART D, DART E, DART F, DART G, DART H, DART I, and DART

1; the anti-PD-1 antibodies: hPD-1 mAb 7(1.2) IgG4 (P), hPD-1 mAb 7(1.2) IgG1 (AA), PD-1 mAb A IgG1 (AA) and PD-1 mAb A IgG4 (P); and the anti-LAG-3 antibodies: hLAG-3 mAb 1(1.4) IgG4 (P), LAG-3 mAb A IgG4 (P), hLAG-3 mAb 1(1.4) IgG1 (AA), and LAG-3 mAb A IgG1 (AA) were examined essentially as described above. The PD-1 x LAG-3 bispecific diabody constructs were tested for both PD-1 and LAG-3 binding, while the anti-PD-1 and anti-LAG-3 antibodies were only tested for binding to their respective antigens. For these studies NSO cells expressing PD-1 or LAG-3 were utilized. The diabodies and antibodies were utilized (170.0-0.013 μM or 85.0-0.0021 μM (four fold serial dilutions). The EC50 and EC90 values were determined and are presented in **Tables 15-16**. The sample mean (SM) and standard deviation (SD **σ**) are provided where 2 or more separate experiments were performed.

Table 15

Molecule	Saturation Binding PD-1			
	EC50 (μM)		EC90 (μM)	
	SM	SD σ	SM	SD σ
DART A	1.9297	0.4324	9.6027	0.4801
DART B	1.7640§		12.2700§	
DART D	2.2267	0.4140	10.9313	2.6351
DART E	3.2180	0.5742	23.840	3.2385
DART F	1.4320§		14.5800§	
DART G	1.1488	0.6227	3.4220	2.4600
DART H	4.5310§		22.6600§	
DART I	1.3232	0.4890	7.8135	4.0821
DART 1	2.1329	1.4850	13.8113	9.0256
hPD-1 mAb 7(1.2) IgG4 (P)	1.2083	0.8112	3.9340	1.8746
PD-1 mAb A IgG4 (P)	2.3470	1.2362	22.7770	15.0690
hPD-1 mAb 7(1.2) IgG1 (AA)	1.0879	0.3958	7.4153	3.0794
PD-1 mAb A IgG1 (AA)	1.6733	0.5464	9.9543	6.6569

§ results from a single experiment

Table 16				
Molecule	Saturation Binding LAG-3			
	EC50 (μM)		EC90 (μM)	
	SM	SD σ	SM	SD σ
DART A	0.8402	0.2231	4.4448	2.4770
DART B	1.0750§		9.8580§	
DART D	0.8985	0.5326	5.7967	4.7329
DART E	0.9250	0.8075	5.6450	5.6809
DART F	5.0090	0.5770	19.3350	4.7447
DART G	0.9396	0.3045	8.5507	4.7448
DART H	2.3840§		9.7810	4.2412
DART I	0.5321	0.0547	4.198	3.2188
DART 1	20.0233	2.1454	115.97	15.2425
hLAG-3 mAb 1(1.4) IgG4 (P)	1.0057	0.1969	5.1360	4.7904
LAG-3 mAb A IgG4 (P)	0.5968	0.1376	2.0833	0.3244
hLAG-3 mAb 1(1.4) IgG1 (AA)	0.6069	0.3430	3.6373	2.4762
LAG-3 mAb A IgG1 (AA)	0.4523	0.1660	2.0187	0.7035

§ results from a single experiment

[00466] The binding saturation studies demonstrate that the PD-1 x LAG-3 bispecific diabody constructs retain binding to PD-1 and have binding profiles that are similar to the binding profiles of the parental anti-PD-1 antibodies. Similarly, the PD-1 x LAG-3 bispecific diabody constructs retain binding to LAG-3 and, with the exception of DART 1, have binding profiles that are similar to the binding profiles of the parental anti-LAG-3 antibodies.

Example 6
PD-1 x LAG-3 Bispecific Molecules Inhibition Studies

[00467] The ability of the PD-1 x LAG-3 bispecific molecules: DART A, DART B, DART D, DART E, DART F, DART G, DART H, DART I, DART 1 and BSAB A; and the anti-PD-1 antibodies: hPD-1 mAb 7(1.2) IgG4 (P), hPD-1 mAb 7(1.2) IgG1 (AA), PD-1 mAb A IgG1 (AA) and PD-1 mAb A IgG4 (P), to block binding of human PD-L1 (shPD-L1) and human PD-L2 (shPD-L2) to PD-1 expressed on the surface of NSO cells was examined essentially as described above. The diabodies and antibodies were utilized at 33.75-0.002 μM or 107.5-0.0001 μM (four fold serial dilutions).

[00468] The IC50 and IC90 values were determined and are presented in **Table 17**. The sample mean (SM) and standard deviation (SD **σ**) are provided where 2 or more separate experiments were performed.

Table 17								
Molecule	block sPD-L1/PD-1 binding				block sPD-L2/PD-1 binding			
	IC50 (μM)		IC90 (μM)		IC50 (μM)		IC90 (μM)	
	SM	SD σ	SM	SD σ	SM	SD σ	SM	SD σ
DART A	0.9645	0.1485	5.6312	1.5247	1.6273	0.4285	6.9335	3.9849
DART B	1.1515	0.0007	4.8615	0.2199	2.1150	0.3154	7.9550	0.0933
DART D	1.5548	0.1692	7.8950	2.5135	3.1255	0.5869	9.2973	5.5426
DART E	1.6533	0.3307	7.8470	1.1642	2.9460	0.7736	6.6135	0.0177
DART F	0.5697	0.1729	2.0360	0.1174	0.8389	0.0846	1.7995	0.2171
DART G	1.6013	0.3581	8.1953	1.5708	2.5540	0.7891	7.4810	0.2333
DART H	3.3950	0.1018	18.640	9.5742	6.2065	3.6847	29.395	3.8679
DART I	0.8363	0.1302	5.3115	0.3125	1.286	0.3125	6.2485	1.3951
DART 1	1.7467	0.3097	5.4533	1.0214	2.8355	1.8250	7.2735	3.9831
BSAB A	2.1590	0.3097	11.075	0.8132	4.8775	0.5438	15.580	1.3294
hPD-1 mAb 7(1.2) IgG4 (P)	0.5186	0.1668	3.8050	1.2227	1.0425	0.2563	3.4880	0.5459
PD-1 mAb A IgG4 (P)	0.9209	0.3256	4.3023	0.7069	1.3859	0.3882	5.1675	0.2943
hPD-1 mAb 7(1.2) IgG1(AA)	0.7320	0.2337	3.2048	1.1479	0.9769	0.2893	2.8437	1.4801
PD-1 mAb A IgG1 (AA)	1.0765	0.2393	5.2775	0.9933	1.9510	0.8814	5.0880	1.3831

[00469] The ligand binding inhibition studies demonstrate that the PD-1 x LAG-3 bispecific diabody constructs retain the ability to inhibit the binding of sPD-L1 and sPD-L2 to PD-1 on the cell surface.

[00470] In addition, the ability of the PD-1 x LAG-3 bispecific molecules: DART A, DART B, DART D, DART E, DART F, DART G, DART H, DART I, DART 1 and BSAB A; and the anti-LAG-3 antibodies: hLAG-3 mAb 1(1.4) IgG4 (P), LAG-3 mAb A IgG4 (P), hLAG-3 mAb 1(1.4) IgG1 (AA), and LAG-3 mAb A IgG1 (AA), to block binding of human LAG-3 to native MHC class II on the surface of Daudi cells was examined. Briefly, each PD-1 x LAG-3 bispecific molecule and control anti-LAG-3 antibody was mixed with a biotinylated-soluble human LAG-3-Fc fusion protein (shLAG-3), (at 0.5 μg/ml) and were separately incubated with MHC II-positive Daudi cells (2.5 x 10^6 cells). The amount of LAG-3 binding to the surface of the Daudi cells was determined using a PE-conjugated Streptavidin secondary antibody by FACS analysis. The diabodies and antibodies were utilized at 27.5-0.026 μM (two fold serial dilutions) or 107.5-0.0001 μM (four fold serial dilutions), or 35-0.002 μM (four fold serial dilutions).

[00471] The IC50 and IC90 values were determined and are presented in **Table 18**. The sample mean (SM) and standard deviation (SD **σ**) are provided where 2 or more separate experiments were performed.

Table 18

Molecule	Block shLAG-3/MHC Class II Binding			
	EC50 (μM)		EC90 (μM)	
	SM	SD σ	SM	SD σ
DART A	1.3835	1.6465	8.396102	8.3962
DART B	0.4081	0.1104	3.0645	0.3924
DART D	1.1843	1.1398	8.0041	7.3317
DART E	3.2706	2.9177	28.9683	24.1694
DART F	1.5347	1.2674	10.3920	11.2555
DART G	2.0618	3.3552	11.4422	12.4964
DART H	2.8967	4.9817	17.2533	21.1420
DART I	0.4864	0.1549	2.339	1.1780
DART 1	15.9610	14.0883	87.1486	109.533
BSAB A	0.7101	0.0571	7.2470	1.0706
hLAG-3 mAb 1(1.4) IgG4 (P)	0.4815	0.2176	3.4837	1.7564
LAG-3 mAb A IgG4 (P)	0.7011	0.1900	2.4232	0.3481
hLAG-3 mAb 1(1.4) IgG1 (AA)	0.3637	0.1409	9.4422	7.9319
LAG-3 mAb A IgG1 (AA)	0.5923	0.3407	2.1451	1.1139

[00472] The ligand binding inhibition studies demonstrate that the PD-1 x LAG-3 bispecific diabody constructs retain the ability to inhibit the binding of a shLAG-3-Fc fusion protein to MHC class II on the cell surface. With the exception of DART 1 the PD-1 x LAG-3 bispecific molecules have similar inhibition profiles as the parental anti-LAG-3 antibodies.

Example 7
Blockade of the PD-1/PD-L1 Checkpoint by PD-1 x LAG-3 Bispecific Molecules

[00473] The ability of the PD-1 x LAG-3 bispecific molecules: DART A, DART B, DART D, DART E, DART F, DART G, DART H, DART I, DART 1 and BSAB A; and the anti-PD-1 antibodies: hPD-1 mAb 7(1.2) IgG4 (P), hPD-1 mAb 7(1.2) IgG1 (AA), PD-1 mAb A IgG1 (AA) and PD-1 mAb A IgG4 (P), to antagonize the PD-1/PD-L1 axis (*i.e.*, block the PD-1/PD-L1 interaction and prevent down-regulation of T-cell responses) was examined in a Jurkat-luc2-NFAT/CHO-PD-L1 luciferase reporter assay (using CHO/PD-L1 cells and MNFAT-luc2/PD-1 Jurkat cells) essentially as described above. The diabodies and antibodies were utilized at 100-0.0065 μM (four fold serial dilutions) or 100-0.0013 μM (five fold serial dilutions).

[00474] The IC50 and IC90 values were determined and are presented in **Table 19.** The sample mean (SM) and standard deviation (SD **σ**) are provided where 2 or more separate experiments were performed.

Table 19

Molecule	Reporter Signaling			
	IC50 (μM)		IC90 (μM)	
	SM	SD σ	SM	SD σ
DART A	0.8804	0.1949	7.9115	1.3232
DART B	1.079	0.1535	7.5413	3.1483
DART D	1.4044	0.2584	12.0786	3.6616
DART E	1.4060	0.1222	13.7867	1.4981
DART F	0.3404	0.0103	1.8710	0.481
DART G	0.6914	0.0206	4.2090	0.7331
DART H	36.6167	20.8078	968.300	811.8471
DART I	1.3335	0.3641	12.146	6.8787
DART 1	11.8807	3.4905	1048.2000	1508.9992
BSAB A	9.7825	1.0288	113.3350	22.2951
hPD-1 mAb 7(1.2) IgG4 (P)	0.6460	0.3035	6.0736	2.5513
PD-1 mAb A IgG4 (P)	1.328	0.7439	16.5138	9.7149
hPD-1 mAb 7(1.2) IgG1(AA)	0.5214	0.1541	4.7592	2.1044
PD-1 mAb A IgG1 (AA)	1.4514	1.0049	35.7382	40.9858

[00475] The reporter signaling studies demonstrate that the majority of the PD-1 x LAG-3 bispecific diabody constructs retain the ability to inhibit the binding of sPD-L1 to PD-1 on the cell surface. The tetravalent PD-1 x LAG-3 bispecific diabody constructs, DART A, DART B, DART D, DART-E, DART F, DART G and DART I were the strongest inhibitors in this assay. Similar results were obtained for several of these bispecific constructs examined in a PD-L2 reporter assay.

Example 8
Functional Activity of PD-1 x LAG-3 Bispecific Molecules

[00476] The ability of PD-1 x LAG-3 bispecific molecules to enhance cytokine release through checkpoint inhibition was examined in SEB-stimulated PBMCs upon restimulation essentially as described above except where noted.

[00477] In initial studies the ability of the PD-1 x LAG-3 bispecific molecules: DART A, DART D, DART E, DART F, DART G, DART H; and the anti-PD-1 and anti-LAG antibodies:

PD-1 mAb A IgG4 (P) and LAG-3 mAb A IgG4 (P), alone or in combination to enhance cytokine release through checkpoint inhibition was examined. In these assays the PD-1 x LAG-3 bispecific molecules and antibodies were used at a total concentration of 3.125, 12.5, or 50 nM, and the PBMCs were stimulated with 0.2 ng/mL of SEB (previous studies used 0.1 ng/mL). For these studies, where a combination of antibodies is used each antibody is provided at one half of the total concentration, (*i.e.*, 1.563, 6.25, or 25 nM). **Figures 16A** and **16B** shows the IFNγ secretion profiles from SEB-stimulated PBMCs from two representative responding donors, D: 35644 and D: 59697, respectively.

[00478] As noted, not all donors respond to SEB at 0.1 or 0.2 ng/mL. To enhance SEB stimulation of PBMCs from a wider number of donors SEB was used at a high concentration of 85 ng/mL, or a middle concentration of 0.5 ng/mL in additional studies. At these concentrations SEB stimulation is more robust across more donors, although donor to donor variability may still be seen.

[00479] In one such study the ability of the PD-1 x LAG-3 bispecific molecules: DART A, DART B; the anti-PD-1 antibody: hPD-1 mAb 7(1.2) IgG4(P); the anti-LAG-3 antibody: LAG-3 mAb 1(1.4) IgG4(P); and the combination of: PD-1 mAb A IgG4 (P) and LAG-3 mAb A IgG4 (P), to enhance cytokine release through checkpoint inhibition was examined. In these assays the PD-1 x LAG-3 bispecific molecules and antibodies were used at a concentration of 0.019, 0.078, 0.3125, 1.25, 5, or 20 nM and the PBMCs were stimulated with 85 ng/mL of SEB. For this assay where a combination of antibodies is used each antibody was provided at the indicated concentration and thus the total antibody concentration is twice the concentration used for each antibody (*i.e.*, 0.038, 0.156, 0.625, 2.5, 10, or 40 nM). **Figures 17A** and **17B** show the IFNγ secretion profiles from SEB-stimulated PBMCs from two representative donors, D: 55515 and D: 54024, respectively.

[00480] In another study the PD-1 x LAG-3 bispecific molecules: DART A, DART B, DART C; the anti-PD-1 antibody: hPD-1 mAb 7(1.2) IgG4(P); the anti-LAG-3 antibody: LAG-3 mAb 1(1.4) IgG4(P); and the combination of: PD-1 mAb A IgG4 (P) and LAG-3 mAb A IgG4 (P), to enhance cytokine release through checkpoint inhibition was examined. In these assays the PD-1 x LAG-3 bispecific molecules and antibodies were used at a total concentration of 0.048, 0.195, 0.78, 3.125, 12.5, or 50 nM and the PBMCs were stimulated with 0.5 ng/mL of SEB. For these studies, where a combination of antibodies is used each antibody is provided at one half of the total concentration (*i.e.*, 0.024, 0.098, 0.39, 1.563, 6.25, or 25 nM). **Figures 18A**

and **18B** show the IFNγ secretion profiles from SEB-stimulated PBMCs from two representative donors, D: 20990 and D: 54947, respectively).

[00481] In a further study, the release of the cytokine IL-2 was examined. Specifically, the PD-1 x LAG-3 bispecific molecules: DART D, DART H; the anti-PD-1 antibodies: PD-1 mAb A IgG4 (P), hPD-1 mAb 7(1.2) IgG4(P); the anti-LAG-3 antibodies: LAG-3 mAb A IgG4 (P) and LAG-3 mAb 1(1.4) IgG4(P); and the combination of: PD-1 mAb A IgG4 (P) and LAG-3 mAb A IgG4 (P), and hPD-1 mAb 7(1.2) IgG4(P) and LAG-3 mAb 1(1.4) IgG4(P), to enhance IL-2 release through checkpoint inhibition was examined. In these assays the PD-1 x LAG-3 bispecific molecules and antibodies were used at a total concentration of 3.125, 12.5, or 50 nM and the PBMCs were stimulated with the high 85 ng/mL concentration of SEB. For these studies, where a combination of antibodies is used each antibody is provided at one half of the total concentration (*i.e.*, 1.563, 6.25, or 25 nM). **Figure 19** shows the IL-2 secretion profile from SEB-stimulated PBMCs from a representative donor (D: 54024).

[00482] In additional studies the PD-1 x LAG-3 bispecific molecules: DART B, and DART I; the anti-PD-1 antibodies: PD-1 mAb A IgG4 (P), and hPD-1 mAb 7(1.2) IgG4(P); the anti-LAG-3 antibodies: LAG-3 mAb A IgG4 (P), hLAG-3 mAb 1(1.4) IgG4(P), and hLAG-3 mAb 6(1.1) IgG4 (P); and the combinations of: PD-1 mAb A IgG4 (P) and LAG-3 mAb A IgG4 (P), hPD-1 mAb 7(1.2) IgG4(P) and hLAG-3 mAb 1(1.4) IgG4(P), and hPD-1 mAb 7(1.2) IgG4(P) and hLAG-3 mAb 6(1.1) IgG4 (P) to enhance cytokine release through checkpoint inhibition was examined. In these assays the PD-1 x LAG-3 bispecific molecules and antibodies were used at a concentration of 0.0061, 0.024, 0.09, 0.39, 1.56, 6.25 or 25 nM and the PBMCs were stimulated with 0.5 ng/mL of SEB. For these studies, where a combination of antibodies is used each antibody is provided at the indicated concentration and thus the total antibody concentration is twice the concentration used for each antibody (*i.e.*, 0.0122, 0.048, 0.18, 0.78, 3.12, 12.5 or 50 nM). **Figure 20** shows the IFNγ secretion profiles from SEB-stimulated PBMCs from a representative donor D: 56041).

[00483] The ability of the PD-1 x LAG-3 bispecific molecule DART I; the combination of the anti-PD-1 antibody PD-1 mAb A IgG4 and the anti-LAG-3 antibody LAG-3 mAb A IgG4 (P); and a negative control antibody to enhance antigen-specific T cell responses was examined using a Tetanus-Toxoid Recall Assay. In particular, the response of antigen-specific enhanced secretion of cytokines was measured using tetanus toxoid as a recall antigen in coculture assay system. Briefly, CD4 memory T cells (0.5 – 1.0 $X10^5$ cells/well) were isolated using negative

selection isolation kits (Miltenyi Biotec, San Diego, CA and Invitrogen, Carlsbad, CA) from human peripheral blood and cultured for 5-7 days with irradiated monocytes (0.01 - 0.05X10^5 cells/well, 3500 rads) from the same donor in the presence or absence of 5 μg/mL the recall antigen tetanus toxoid (TTd) and dilution (starting at 25nM) of DART I, PD-1 mAb A IgG4 + LAG-3 mAb A IgG4(P), or an isotype control. In parallel plates, proliferation was measured through the incorporation of tritiated thymidine and IL-2 and IFNγ was measured using ELISA (R&D systems, Minneapolis, MN) at days 5-7. **Figures 21A-D** show the IFNγ (**Figure 21A, 21C**) and IL-2 (**Figure 21B, 21D**) secretion profiles at day 7, from two representative donors (D50702 and D54267).

[00484] The results of these studies demonstrate that the PD-1 x LAG-3 bispecific molecules dramatically enhanced IFNγ (**Figures 16A-16B, 17A-17B, 18A-18B**, **20**), and IL-2 (**Figure 19**) production from SEB-stimulated PBMCs upon restimulation. In addition, the PD-1 x LAG-3 bispecific molecules dramatically enhanced IFNγ production (**Figures 21A and 21C**) from CD4 memory T cells stimulated with tetanus toxoid. In particular, the tetravalent PD-1 x LAG-3 bispecific molecules provided a greater enhancement than the combination of anti-PD-1 antibodies with anti-LAG-3 antibodies.

Example 9
Pharmacokinetics of PD-1 x LAG-3 Bispecific Molecules

[00485] The pharmacokinetics of a representative PD-1 x LAG-3 bispecific molecule, DART I and a representative anti-PD-1 antibody, PD-1 mAb A were examined in Cynomolgus monkeys. Briefly, two cynomolgus monkeys (one male and one female) were infused with a single dose of DART I (5 mg/kg) or PD-1 mAb A (10 mg/kg) and the serum concentration of the molecules was monitored over time using a sandwich ELISA assay. Briefly, maxisorb 96-well assay plates were coated with soluble human PD-1 (shPD-1), blocked with bovine serum albumin, washed and incubated with calibration standards, quality control standards and diluted serum samples. The amount of captured DART I and PD-1 mAb A was assessed by the sequential addition of a goat anti-human IgG Fc-biotin secondary and streptavidin-horseradish peroxidase (SA-HRP). HRP activity was detected using TMB substrate. All samples were analyzed a microplate reader (SpectraMax M2e, Molecular Device, Sunnyvale, CA) and the OD signals produced by the standard calibrators were used in the four-parameter logistic model using SoftMax Pro software (Version 5.4, Molecular Devices). The concentrations of PD-1 mAb A, or DART I were determined from the interpolation of the

samples' OD signal data with the equation describing the standard curve. The lower limit of quantitation (LLOQ) for this assay was estimated at 9.775 ng/mL.

[00486] **Figure 22** shows the serum concentration over time, the lines represents the mean of both male (filled symbols) and female (open symbols) monkeys infused with DART I (solid line, triangles) or PD-1 mAb A (dashed line, circles). These data demonstrate that the pharmacokinetics of a PD-1 x LAG-3 bispecific molecule are comparable to those of an anti-PD-1 antibody in cynomolgus monkeys.

Example 10
Toxicology Study of PD-1 Antibodies and PD-1 x LAG-3 Bispecific Molecules

[00487] The safety profile of a representative a representative anti-PD1 antibody, hPD-1 mAb 7 (1.2) IgG4 (P), and a representative PD1 x LAG3 bispecific molecule, DART I, was assessed in a non-GLP (Good Laboratory Practice) dosing study in cynomolgus monkeys.

[00488] In this study the potential toxicity and toxicokinetics of the anti-PD-1 antibody (hPD-1 mAb 7 (1.2) IgG4 (P)), when administered by multiple intravenous infusions was evalulated. In addition, the potential toxicity and pharmacokinetics of the PD-1 x LAG-3 DART molecule (DART I), when administered by single intravenous infusion was also evaluated. The study design is presented in **Table 20**.

Table 20

Group No.	Test Material	Dose Level (mg/kg)	Dosing Days	Dose Volume	Dose (mg/mL)	No. of Animals	
						Males	Females
1	Control	0	1, 8, 15	5	0	1[a]	1[a]
2A	hPD-1 mAb 7 (1.2) IgG4 (P)	1	1, 8, 15	5	0.2	1[a]	1[a]
2B	hPD-1 mAb 7 (1.2) IgG4 (P)	1	1, 8, 15	5	0.2	1[b]	1[b]
3A	hPD-1 mAb 7 (1.2) IgG4 (P)	100	1, 8, 15	5	20	1[a]	1[a]
3B	hPD-1 mAb 7 (1.2) IgG4 (P)	100	1, 8, 15	5	20	1[b]	1[b]
4	DART I	5	1	5	1	1[c]	1[c]

[a] Groups 1, 2A, and 3A were dosed beginning on Day 1 and necropsied 72 hours following their last (third) dose on Day 18.
[b] Groups 2B and 3B were dosed beginning on Day 1 and necropsied 7 days following their last (third) dose on Day 22.
[c] Group 4 was dosed beginning on Day 1 and followed for 28 days post single dose administration (to Day 29); animals were then returned to colony.

[00489] The following parameters and endpoints were evaluated in this study: clinical signs, body weights, food consumption, body temperature, clinical pathology parameters (hematology, coagulation, and clinical chemistry), bioanalysis and toxicokinetic parameters, anti-drug antibody analysis, flow cytometry, cytokine, gross necropsy findings, organ weights, and histopathologic examinations.

[00490] All animals survived until scheduled euthanasia on Day 18 or 22 or release from study on Day 29. For hPD-1 mAb 7 (1.2) IgG4 (P) there were no test article-related changes in clinical signs, food consumption, body weights, body temperature, hematology, coagulation, or clinical chemistry parameters, or gross necropsy findings. At Days 18 and 22, increases in spleen weight and a dose-dependent mild to moderate lymphohistiocytic infiltrate of the red pulp were evident in animals receiving hPD-1 mAb 7 (1.2) IgG4 (P) at 1 or 100 mg/kg. As compared to surrounding lymphocytes, the lymphohistiocytic cells had pale cytoplasm and irregular nuclei. Rare mitotic figures were evident. The infiltrate was a microscopic correlate for the increased spleen weight.

[00491] The serum concentration-time profiles for animals given hPD-1 mAb 7 (1.2) IgG4 (P) show the profile expected for an antibody in this species, with a few exceptions. The slopes of the curves after the third dose dropped more sharply than after the first dose for two animals in the 1 mg/kg dose group and two animals in the 100 mg/kg dose group, indicating the possible emergence of anti-drug antibodies (ADA) at the later cycles. Analysis showed that 2/4 animals developed ADA in the 1 mg/kg group and 1/4 animals developed ADA in the 100 mg/kg group.

[00492] In conclusion, administration of hPD-1 mAb 7 (1.2) IgG4 (P) by intravenous infusion once weekly for 3 weeks (Days 1, 8, and 15) was well-tolerated in cynomolgus monkeys at levels of 1 and 100 mg/kg. A dose-dependent mild to moderate lymphohistiocytic cellular infiltrate of the splenic red pulp was present at 1 and 100 mg/kg hPD-1 mAb 7 (1.2) IgG4 (P).

[00493] For DART I, there were no test article-related changes in clinical signs, food consumption, body weights, body temperature, hematology, or coagulation parameters. DART I-related changes in clinical chemistry parameters included non-adverse, transient elevations in aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) on Day 2. The average AST change was 3.2x vehicle-treated control animals and 7.8x prestudy levels, with levels above the control reference range2. The average LDH change was 2.5x vehicle-treated control

animals and 6.9x prestudy levels. Both parameters returned to near baseline levels on Day 8. In conclusion, single administration of DART-I by intravenous infusion was well tolerated in cynomolgus monkeys at a level of 5 mg/kg.

Example 11
Single Dose PK Study with anti-PD-1 Antibodies

[00494] A single-dose PK study with selected toxicological endpoints was conducted in cynomolgus monkeys. In this study, hPD-1 mAb 7 (1.2) IgG4 (P) was compared to two other anti-PD1 IgG4 (P), κ mAbs: PD-1 mAb A IgG4 (P) and PD-1 mAb B IgG4 (P). Each antibody was administered at 10 mg/kg by 1-hour intravenous infusion to 2 monkeys (1M, 1F) and animals were monitored for 65 days.

[00495] There were no test article-related clinical signs, changes in body weight, food consumption, cytokine, or immunophenotyping associated with administration of hPD-1 mAb 7 (1.2) IgG4 (P) or PD-1 mAb A IgG4 (P). Data were similar for PD-1 mAb B IgG4 (P)with the exception that elevations in IL-5 were observed following PD-1 mAb B IgG4 (P) administration.

[00496] Anti-PD-1 antibody binding to PD-1 on the surface of T cells was determined by flow cytometry using a competition method in which the mean fluorescence intensity (MFI) of fluorescently labeled hPD-1 mAb 7 (1.2) IgG4 (P) binding to T cells in the absence (PBS control) or presence of excess competitor (unlabeled hPD-1 mAb 7 (1.2) IgG4 (P)) for the full time course of blood samples collected from the cynomolgus monkeys treated with hPD-1 mAb 7 (1.2) IgG4 (P), PD-1 mAb A IgG4 (P) or PD-1 mAb B IgG4 (P). As shown in **Figures 23A-23C**, hPD-1 mAb 7 (1.2) IgG4 (P) and PD-1 mAb B IgG4 (P) demonstrated prolonged binding to PD-1 on the surface of CD4+ and CD8+ T cells (PD-1 binding maintained at ≥ 80% for 28 days or more) (**Figures 23A and 23C**, respectively) compared to PD-1 mAb A IgG4 (P) (PD-1 binding maintained at ≥ 80% for 21 days or less) (**Figure 23B**). For each of the anti-PD-1 antibodies, the T-cell PD-1 binding data correlated with their serum concentrations.

Example 12
Repeat Dose Toxicology Studies

[00497] To assess the safety, toxicokinetic, and pharmacodynamic profile of the therapeutic molecules of the present invention, an exemplary molecule (hPD-1 mAb 7 (1.2) IgG4 (P)) was administered to cynomolgus monkeys and a GLP (Good Laboratory Practice) dosing study was performed. In this study, four groups of animals (10 per group, 5 males, and 5 females) were

treated with hPD-1 mAb 7 (1.2) IgG4 (P) or a control article, once weekly by infusion at 3 dose levels. The animals were evaluated for any potential toxicity during a 4-week drug dosing period followed by monitoring during an additional 10-week drug-free period. The experimental design of this study is presented in **Table 21**. Animals were dosed once weekly via a one-hour intravenous infusion using a calibrated infusion pump on Study Days 1, 8, 15, and 22. One male and one female from each group were sacrificed on Day 25, the remaining animals were sacrificed on Study Day 95. The effects of hPD-1 mAb 7 (1.2) IgG4 (P) administration on the leukocyte subpopulations in circulation, including the occupancy of PD-1 receptors on T-lymphocytes were assessed. In addition, the anti-drug antibody (ADA) profiles were determined.

Table 21

Group No.	Test Material[a]	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose (mg/mL)	No. of Animals[b]			
					Main Study		Recovery Study	
					M	F	M	F
1	Control	0	5.88	0	3	3	2	2
2	hPD-1 mAb 7 (1.2) IgG4 (P)	10	5.88	1.7	3	3	2	2
3	hPD-1 mAb 7 (1.2) IgG4 (P)	40	5.88	6.8	3	3	2	2
4	hPD-1 mAb 7 (1.2) IgG4 (P)	150	5.88	25.5	3	3	2	2

[a] Control and hPD-1 mAb 7 (1.2) IgG4 (P) were administered weekly via intravenous infusion

[b] Six monkeys (3M/3F) per group were necropsied on Day 25, while the remaining recovery group monkeys (2M/2F) were necropsied on Day 95

[00498] Once weekly intravenous (IV) infusions of hPD-1 mAb 7 (1.2) IgG4 (P) at 0, 10, 40, and 150 mg/kg in cynomolgus monkeys were well tolerated and all animals survived to their scheduled euthanasia on Days 25 or 95. There were no hPD-1 mAb 7 (1.2) IgG4 (P)-related changes in clinical signs, food consumption, body weights, physical, ophthalmic, and neurological examinations, electrocardiology, body temperatures, respiratory rates, blood pressure and heart rates, coagulation, clinical chemistry, and urinalysis parameters, organ weights, or gross necropsy findings.

[00499] hPD-1 mAb 7 (1.2) IgG4 (P)-related changes in hematology parameters included transient decreases in lymphocyte titers. Lymphocyte titers were moderately decreased compared to pre-study (Day 1 predose) on Day 2 (23 hours post infusion) in males and females at $\geq$ 10 mg/kg, statistically significant for males at 10 and 40 mg/kg and females at 40 and 150

mg/kg compared to controls. Lymphocyte titers returned to near prestudy levels on Day 8 predose but were mildly decreased for some individual males and females at all dose levels (0.47x to 0.68x prestudy) on Day 9 (23 hours post infusion). Lymphocyte titers increased prior to dosing on Days 15 and 22, but decreased for some individual males and females (0.36x to 0.54x prestudy) on Days 16 and 23 (23 hours post infusion).

[00500] A dose-independent, transient decline in circulating immune cell populations, including total leukocytes, T cells, B cells, and NK cells, was observed 23 hours following the end of infusion in hPD-1 mAb 7 (1.2) IgG4 (P)-treated animals compared with the control group. The largest magnitude in change was observed following the first dose administration on Day 1; smaller magnitude changes were transiently observed following subsequent doses on Days 8, 15, or 22. Immune cell populations generally recovered to at or near baseline values by 72 hours post-EOI and throughout the recovery phase. No changes in circulating monocytes were observed in hPD-1 mAb 7 (1.2) IgG4 (P)-treated animals compared with the control group.

[00501] Maximal hPD-1 mAb 7 (1.2) IgG4 (P) binding to PD-1+/CD4+ and PD-1+/CD8+ cells was observed during the hPD-1 mAb 7 (1.2) IgG4 (P) treatment phase of the study at all doses tested (10, 40 or 150 mg/kg). In recovery, animals that did not develop anti-drug antibody (ADA) responses, serum hPD-1 mAb 7 (1.2) IgG4 (P) concentrations remained above 29 μg/mL and maximal hPD-1 mAb 7 (1.2) IgG4 (P) binding to PD-1+/CD4+ and PD-1+/CD8+ T cells was maintained during the entire 10-week recovery period. In these animals, there was no evidence of PD-1 modulation on the T cells. In recovery animals that developed ADA responses, the frequency of MGD012-bound PD-1+ T cells declined to baseline levels. The declines from maximal hPD-1 mAb 7 (1.2) IgG4 (P) binding on PD-1+/CD4+ and PD-1+/CD8+ cells of ADA-positive animals generally occurred when the apparent serum hPD-1 mAb 7 (1.2) IgG4 (P) concentrations dropped below approximately 25 μg/mL. However, it is not known if this apparent threshold relationship applies to ADA-negative animals, since the presence of ADA in ADA-positive animals may contribute to blocking the binding of PD-1 antibodies to PD-1.

[00502] There were minimal sex-associated differences in the pharmacokinetic responses of hPD-1 mAb 7 (1.2) IgG4 (P), which were linear across the dose range evaluated (10 to 150 mg/kg). For hPD-1 mAb 7 (1.2) IgG4 (P) at 10, 40, and 150 mg/kg, the gender combined mean C_{max} was 240 μg/mL (0.240 mg/mL), 1078 μg/mL (1.08 mg/mL), and 3938 μg/mL

(3.94 mg/mL) and the AUC was 47310 h•μg/mL (47.3 h•mg/mL), 205723 h•μg/mL (206 h•mg/mL), and 745681 h•μg/mL (746 h•mg/mL), respectively. Mean clearance by non-compartmental analysis (NCA) of the first cycle of hPD-1 mAb 7 (1.2) IgG4 (P) before the emergence of ADA, was 0.21 mL/h/kg, substantially lower than the glomerular filtration rate of cynomolgus monkeys, as would be expected for a large molecular weight protein. Mean steady-state volume of distribution by NCA of the first cycle of hPD-1 mAb 7 (1.2) IgG4 (P) was 68 mL/kg, approximately 1.5 times the serum volume, but less than the extracellular water space. This suggests that hPD-1 mAb 7 (1.2) IgG4 (P) extravasates from the vascular compartment into the tissue extracellular space, but that not all of the extracellular space was accessible to this molecule. The average value of the mean residence time (MRT) by NCA of the first cycle of hPD-1 mAb 7 (1.2) IgG4 (P) was 335 hours or approximately 14 days. Emergence of ADA decreased the concentrations of hPD-1 mAb 7 (1.2) IgG4 (P) in Cycles 2 to 4. Evidence of decreased hPD-1 mAb 7 (1.2) IgG4 (P) serum concentrations following repeated doses of hPD-1 mAb 7 (1.2) IgG4 (P) were observed in 7/10, 4/10, and 3/10 animals in the 10, 40, and 150 mg/kg dose groups, respectively. The presence of ADA against hPD-1 mAb 7 (1.2) IgG4 (P) was confirmed in 4, 2, and 1 of these animals in the 10, 40, and 150 mg/kg dose groups, respectively; all the animals in which ADA was not confirmed were in the terminal necropsy group during which hPD-1 mAb 7 (1.2) IgG4 (P) serum concentrations likely interfered with the ability to detect ADA. Accordingly, in subsequent TK analysis, when a trough concentration was lower than the preceding trough concentration, data from this time forward were censored. From two-compartment modeling of data across all cycles for the 3 dose groups, excluding points that were affected by ADA, mean values for the primary TK parameters for a 2-compartment model were 0.22 mL/h/kg for clearance, 38.5 mL/kg for initial volume of distribution (V_1), and 33.8 mL/kg for V_2, which yielded a mean steady-state volume of distribution (V_{ss}) of 72.3 mL/kg, and an MRT of 329 hours. These values were consistent with parameters obtained from NCA of the first dose. In the absence of ADA, simulations predict that with weekly dosing, steady state would be achieved in cynomolgus monkeys after the 5th dose and the accumulation index would be 2.4.

[00503] On Day 25, hPD-1 mAb 7 (1.2) IgG4 (P)-related minimal multifocal perivascular mononuclear cell infiltrates were present in the superficial dermis of the IV injection site in males at ≥ 40 mg/kg and in females at ≥ 10 mg/kg and were an expected reaction to repeated injection of a foreign protein (monoclonal antibody). On Day 95, there were no hPD-1 mAb 7

(1.2) IgG4 (P)-related microscopic changes noted, indicating recovery of the test article-related change present on Day 25.

[00504] In summary, the results of this study indicate that administration of hPD-1 mAb 7 (1.2) IgG4 (P) via intravenous infusion once weekly (Days 1, 8, 15, and 22) was clinically well tolerated in cynomolgus monkeys at levels of 10, 40, or 150 mg/kg. Effects observed were limited to transient decreases in circulating lymphocytes and minimal injection-site changes related to injection of a foreign protein. Based on these results, the no-observed-adverse-effect level (NOAEL) was considered to be 150 mg/kg (gender combined mean C_{max} of 3.94 mg/mL and AUC of 746 h•mg/mL).

[00505] All publications and patents mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference in its entirety. While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth.

What Is Claimed Is:

Claim 1. An anti-human PD-1-binding molecule that comprises a Variable Heavy Chain Domain and a Variable Light Chain Domain, wherein:

said Variable Heavy Chain Domain comprises a CDR_H1 Domain, a CDR_H2 Domain and a CDR_H3 Domain, and said Variable Light Chain Domain comprises a CDR_L1 Domain, a CDR_L2 Domain, and a CDR_L3 Domain, wherein:

(A) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of PD-1 mAb 1, and respectively have the amino acid sequences: **SEQ ID NO:71**, **SEQ ID NO:72**, and **SEQ ID NO:73**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of PD-1 mAb 1, and respectively have the amino acid sequences: **SEQ ID NO:76**, **SEQ ID NO:77**, and **SEQ ID NO:78**;

or

(B) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of PD-1 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:85**, **SEQ ID NO:86**, and **SEQ ID NO:87**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of PD-1 mAb 2, and, respectively have the amino acid sequences: **SEQ ID NO:90**, **SEQ ID NO:91**, and **SEQ ID NO:92**;

or

(C) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of PD-1 mAb 3, and respectively have the amino acid sequences: **SEQ ID NO:99**, **SEQ ID NO:100**, and **SEQ ID NO:101**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of PD-1 mAb 3, and, respectively have the amino acid sequences: **SEQ ID NO:104**, **SEQ ID NO:105**, and **SEQ ID NO:106**;

or

(D) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of PD-1 mAb 4, and respectively have the amino acid sequences: **SEQ ID NO:109**, **SEQ ID NO:110**, and **SEQ ID NO:111**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of PD-1 mAb 4, and, respectively have the amino acid sequences: **SEQ ID NO:114**, **SEQ ID NO:115**, and **SEQ ID NO:116**;

or

(E) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of PD-1 mAb 5, and respectively have the amino acid sequences: **SEQ ID NO:119**, **SEQ ID NO:120**, and **SEQ ID NO:121**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of PD-1 mAb 5, and, respectively have the amino acid sequences: **SEQ ID NO:124**, **SEQ ID NO:125**, and **SEQ ID NO:126**;

or

(F) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of PD-1 mAb 6, and respectively have the amino acid sequences: **SEQ ID NO:129**, **SEQ ID NO:130**, and **SEQ ID NO:131**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of PD-1 mAb 6, and, respectively have the amino acid sequences: **SEQ ID NO:134**, **SEQ ID NO:135**, and **SEQ ID NO:136**;

or

(G) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of PD-1 mAb 7, and respectively have the amino acid sequences: **SEQ ID NO:139**, **SEQ ID NO:140**, and **SEQ ID NO:141**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of PD-1 mAb 7, and, respectively have

the amino acid sequences: **SEQ ID NO:144**, **SEQ ID NO:145**, and **SEQ ID NO:146**;

or

(H) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of PD-1 mAb 8, and respectively have the amino acid sequences: **SEQ ID NO:161**, **SEQ ID NO:162**, and **SEQ ID NO:163**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of PD-1 mAb 8, and, respectively have the amino acid sequences: **SEQ ID NO:166**, **SEQ ID NO:167**, and **SEQ ID NO:168**;

or

(I) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of PD-1 mAb 9, and respectively have the amino acid sequences: **SEQ ID NO:171**, **SEQ ID NO:172**, and **SEQ ID NO:173**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of PD-1 mAb 9, and, respectively have the amino acid sequences: **SEQ ID NO:176**, **SEQ ID NO:177**, and **SEQ ID NO:178**;

or

(J) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of PD-1 mAb 10, and respectively have the amino acid sequences: **SEQ ID NO:192**, **SEQ ID NO:193**, and **SEQ ID NO:194**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of PD-1 mAb 10, and, respectively have the amino acid sequences: **SEQ ID NO:197**, **SEQ ID NO:198**, and **SEQ ID NO:199**;

or

(K) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of PD-1 mAb 11, and respectively have the amino acid sequences: **SEQ ID NO:202**, **SEQ ID NO:203**, and **SEQ ID NO:204**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of PD-1 mAb 11, and, respectively have the amino acid sequences: **SEQ ID NO:207**, **SEQ ID NO:208**, and **SEQ ID NO:209**;

or

(L) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of PD-1 mAb 12, and respectively have the amino acid sequences: **SEQ ID NO:212**, **SEQ ID NO:213**, and **SEQ ID NO:214**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of PD-1 mAb 12, and, respectively have the amino acid sequences: **SEQ ID NO:217**, **SEQ ID NO:218**, and **SEQ ID NO:219**

or

(M) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of PD-1 mAb 13, and respectively have the amino acid sequences: **SEQ ID NO:222**, **SEQ ID NO:223**, and **SEQ ID NO:224**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of PD-1 mAb 13, and, respectively have the amino acid sequences: **SEQ ID NO:227**, **SEQ ID NO:228**, and **SEQ ID NO:229**;

or

(N) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of PD-1 mAb 14, and respectively have the amino acid sequences: **SEQ ID NO:232**, **SEQ ID NO:233**, and **SEQ ID NO:234**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of PD-1 mAb 14, and, respectively have the amino acid sequences: **SEQ ID NO:237**, **SEQ ID NO:238**, and **SEQ ID NO:239**;

or

(O) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of PD-1 mAb 15, and respectively have the amino acid sequences: **SEQ ID NO:242**, **SEQ ID NO:243**, and **SEQ ID NO:244**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of PD-1 mAb 15, and, respectively have the amino acid sequences: **SEQ ID NO:247**, **SEQ ID NO:248**, and **SEQ ID NO:249**;

or

(P) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of hPD-1 mAb 7(1.2), and respectively have the amino acid sequences: **SEQ ID NO:139**, **SEQ ID NO:140**, and **SEQ ID NO:141**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of hPD-1 mAb 7(1.2), and, respectively have the amino acid sequences: **SEQ ID NO:157**, **SEQ ID NO:145**, and **SEQ ID NO:146**;

or

(Q) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of hPD-1 mAb 7(1.3), and respectively have the amino acid sequences: **SEQ ID NO:139**, **SEQ ID NO:140**, and **SEQ ID NO:141**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of hPD-1 mAb 7(1.3), and, respectively have the amino acid sequences: **SEQ ID NO:157**, **SEQ ID NO:158**, and **SEQ ID NO:145**;

or

(R) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of hPD-1 mAb 9(2.2), and respectively have the amino acid sequences: **SEQ ID NO:183**, **SEQ ID NO:172**, and **SEQ ID NO:173**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of hPD-1 mAb 9(2.2), and, respectively

have the amino acid sequences: **SEQ ID NO:188**, **SEQ ID NO:189**, and **SEQ ID NO:178**.

Claim 2. The anti-human PD-1-binding molecule of claim 1, wherein said molecule is an antibody.

Claim 3. The anti-human PD-1-binding molecule of claim 2, wherein said molecule is a chimeric antibody or a humanized antibody.

Claim 4. The anti-human PD-1-binding molecule of any one of claims 1 or 3, wherein said molecule comprises a Heavy Chain Variable Domain having the amino acid sequence of **SEQ ID NO:79**, **SEQ ID NO:93**, **SEQ ID NO:147**, **SEQ ID NO:149**, **SEQ ID NO:179**, **SEQ ID NO:181**, or **SEQ ID NO:250**.

Claim 5. The anti-human PD-1-binding molecule of any one of claims 1, 3 or 4, wherein said molecule comprises a Light Chain Variable Domain having the amino acid sequence of **SEQ ID NO:81**, **SEQ ID NO:95**, **SEQ ID NO:151**, **SEQ ID NO:153**, **SEQ ID NO:155**, **SEQ ID NO:184**, **SEQ ID NO:186**, or **SEQ ID NO:251**.

Claim 6. The anti-human PD-1-binding molecule of any one of claims 1-5, wherein said molecule is a bispecific binding molecule, capable of simultaneously binding to human PD-1 and to a second epitope.

Claim 7. The anti-human PD-1-binding molecule of claim 6, wherein said second epitope is an epitope of a molecule involved in regulating an immune check point present on the surface of an immune cell.

Claim 8. The anti-human PD-1-binding molecule of claim 6, wherein said second epitope is an epitope of B7-H3, B7-H4, BTLA, CD40, CD40L, CD47, CD70, CD80, CD86, CD94, CD137, CD137L, CD226, CTLA-4, Galectin-9, GITR, GITRL, HHLA2, ICOS, ICOSL, KIR, LAG-3, LIGHT, MHC class I or II, NKG2a, NKG2d, OX40, OX40L, PD1H, PD-1, PD-L1, PD-L2, PVR, SIRPa, TCR, TIGIT, TIM-3 or VISTA.

Claim 9. The anti-human PD-1-binding molecule of claim 8, wherein said second epitope is an epitope of CD137, CTLA-4, LAG-3, OX40, TIGIT, or TIM-3.

Claim 10. The anti-human PD-1-binding molecule of claim 9, comprising a LAG-3 epitope-binding site, wherein said LAG-3 epitope-binding site comprises:

(A) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain of the Variable Heavy Chain of LAG-3 mAb 1, having the amino acid sequences: **SEQ ID NO:42**, **SEQ ID NO:43**, and **SEQ ID NO:44**, respectively; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain of the Variable Light Chain of LAG-3 mAb 1, having the amino acid sequences: **SEQ ID NO:46**, **SEQ ID NO:47**, and **SEQ ID NO:48**, respectively;

or

(B) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain of the Variable Heavy Chain of hLAG-3 mAb 1 VH1, having the amino acid sequences: **SEQ ID NO:42**, **SEQ ID NO:43**, and **SEQ ID NO:44**, respectively; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain of the Variable Light Chain of LAG-3 mAb 1 VL4, having the amino acid sequences: **SEQ ID NO:55**, **SEQ ID NO:47**, and **SEQ ID NO:48**, respectively;

or

(C) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain of the Variable Heavy Chain of LAG-3 mAb 6, having the amino acid sequences: **SEQ ID NO:57**, **SEQ ID NO:58**, and **SEQ ID NO:59**, respectively; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain of the Variable Light Chain of LAG-3 mAb 6, having the amino acid sequences: **SEQ ID NO:61**, **SEQ ID NO:62**, and **SEQ ID NO:63**, respectively;

or

(D) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain of the Variable Heavy Chain of hLAG-3 mAb 6 VH1, having the amino acid sequences: **SEQ ID NO:57**, **SEQ ID NO:58**, and **SEQ ID NO:59**, respectively; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain of the Variable Light Chain of LAG-3 mAb 6, having the amino acid sequences: **SEQ ID NO:298**, **SEQ ID NO:62**, and **SEQ ID NO:63**, respectively.

Claim 11. The anti-human PD-1-binding molecule of any one of claims 6-10, wherein said molecule is:

(A) a diabody, said diabody being a covalently bonded complex that comprises two, or three, four or five polypeptide chains; or

(B) a trivalent binding molecule, said trivalent binding molecule being a covalently bonded complex that comprises three, four or five polypeptide chains, or

(C) a bispecific antibody.

Claim 12. The anti-human PD-1-binding molecule of claim 11, wherein said molecule is a diabody and comprises an Albumin-Binding Domain (ABD).

Claim 13. The anti-human PD-1-binding molecule of any one of claims 1-11, wherein said molecule comprises an Fc Region.

Claim 14 The anti-human PD-1-binding molecule of claim 13, wherein said Fc Region, is of the IgG1, IgG2, IgG3, or IgG4 isotype.

Claim 15 The anti-human PD-1-binding molecule of claim 14, wherein said molecule further comprises a Hinge Domain.

Claim 16 The anti-human PD-1-binding molecule of claim 15, wherein said Fc Region and said Hinge Doman are of the IgG4 isotype, and wherein said Hinge Domain comprises a stabilizing mutation.

Claim 17 The anti-human PD-1-binding molecule of any one of claims 13-15, wherein said molecule is an antibody comprising:

(a) SEQ ID NOs: 264 and 265; or

(b) SEQ ID NOs: 264 and 267.

Claim 18. The anti-human PD-1-binding molecule of any one of claims 13-16, wherein said Fc Region is a variant Fc Region that comprises:

(a) one or more amino acid modifications that reduces the affinity of the variant Fc Region for an FcγR; and/or

(b) one or more amino acid modifications that enhances the serum half-life of the variant Fc Region.

Claim 19. The anti-human PD-1-binding molecule of claim 14, wherein said modifications that reduces the affinity of the variant Fc Region for an FcγR comprise the substitution of L234A; L235A; or L234A and L235A, wherein said numbering is that of the EU index as in Kabat.

Claim 20. The anti-human PD-1-binding molecule of claim 14 or 15, wherein said modifications that that enhances the serum half-life of the variant Fc Region comprise the substitution of M252Y; M252Y and S254T; M252Y and T256E; M252Y, S254T and T256E; or K288D and H435K, wherein said numbering is that of the EU index as in Kabat.

Claim 21. The anti-human PD-1-binding molecule of any one of claims 11, 13-16, or 18-20, wherein said molecule is a diabody comprising:

(a) SEQ ID NO:267, wherein X_1 is Ala; X_2 is Tyr; X_3 is Thr; X_4 is Glu, and SEQ ID NO:268; or

(b) SEQ ID NO:267, wherein X_1 is Gly; X_2 is Tyr; X_3 is Thr; X_4 is Glu, and SEQ ID NO:268; or

(c) SEQ ID NO:267, wherein X_1 is Gly; X_2 is Met; X_3 is Ser; X_4 is Thr, and SEQ ID NO:268; or

(d) SEQ ID NOs:269 and 270; or

(e) SEQ ID NOs:271 and 272; or

(f) SEQ ID NOs:273, 274, 275, and 276; or

(g) SEQ ID NOs:277, 278, 279, and 280; or

(h) SEQ ID NOs:281, 282, and 283; or

(i) SEQ ID NOs:290 and 291; or

(j) SEQ ID NOs:292 and 293.

Claim 22. The anti-human PD-1-binding molecule of any one of claims 1-21, wherein said molecule is used to stimulate a T-cell mediate immune response of a subject in need thereof.

Claim 23. The anti-human PD-1-binding molecule of any one of claims 1-21, wherein said molecule is used in the treatment of a disease or condition associated with a suppressed immune system.

Claim 24. The anti-human PD-1-binding molecule of claim 23, wherein the disease or condition is cancer or an infection.

Claim 25. The anti-human PD-1-binding molecule of claim 24, wherein said cancer is characterized by the presence of a cancer cell selected from the group consisting of a cell of: an adrenal gland tumor, an AIDS-associated cancer, an alveolar soft part sarcoma, an astrocytic tumor, bladder cancer, bone cancer, a brain and spinal cord cancer, a metastatic brain tumor, a breast cancer, a carotid body tumors, a cervical cancer, a chondrosarcoma, a chordoma, a chromophobe renal cell carcinoma, a clear cell carcinoma, a colon cancer, a colorectal cancer, a cutaneous benign fibrous histiocytoma, a desmoplastic small round cell tumor, an ependymoma, a Ewing's tumor, an extraskeletal myxoid chondrosarcoma, a fibrogenesis imperfecta ossium, a fibrous dysplasia of the bone, a gallbladder or bile duct cancer, gastric cancer, a gestational trophoblastic disease, a germ cell tumor, a head and neck cancer, hepatocellular carcinoma, an islet cell tumor, a Kaposi's Sarcoma, a kidney cancer, a leukemia, a lipoma/benign lipomatous tumor, a liposarcoma/malignant lipomatous tumor, a liver cancer, a lymphoma, a lung cancer, a medulloblastoma, a melanoma, a meningioma, a multiple endocrine neoplasia, a multiple myeloma, a myelodysplastic syndrome, a neuroblastoma, a neuroendocrine tumors, an ovarian cancer, a pancreatic cancer, a papillary thyroid carcinoma, a parathyroid tumor, a pediatric cancer, a peripheral nerve sheath tumor, a phaeochromocytoma, a pituitary tumor, a prostate cancer, a posterious uveal melanoma, a rare hematologic disorder, a renal metastatic cancer, a rhabdoid tumor, a rhabdomysarcoma, a sarcoma, a skin cancer, a soft-tissue sarcoma, a squamous cell cancer, a stomach cancer, a synovial sarcoma, a testicular cancer, a thymic carcinoma, a thymoma, a thyroid metastatic cancer, and a uterine cancer.

Claim 26. The anti-human PD-1-binding molecule of claim 25, wherein said cancer is colorectal cancer, hepatocellular carcinoma, glioma, kidney cancer, breast cancer, multiple myeloma, bladder cancer, neuroblastoma; sarcoma, non-Hodgkin's lymphoma, non-small cell lung cancer, ovarian cancer, pancreatic cancer, rectal cancer, acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), acute B lymphoblastic leukemia (B-ALL), chronic lymphocytic leukemia (CLL), hairy cell leukemia (HCL), blastic plasmacytoid dendritic cell neoplasm (BPDCN), non-Hodgkin's lymphomas (NHL), including mantel cell leukemia (MCL), and small lymphocytic lymphoma (SLL), Hodgkin's lymphoma, systemic mastocytosis, or Burkitt's lymphoma.

Claim 27. The anti-human PD-1-binding molecule of any one of claims 1-21, wherein said molecule is detectably labeled and is used in the detection of PD-1.

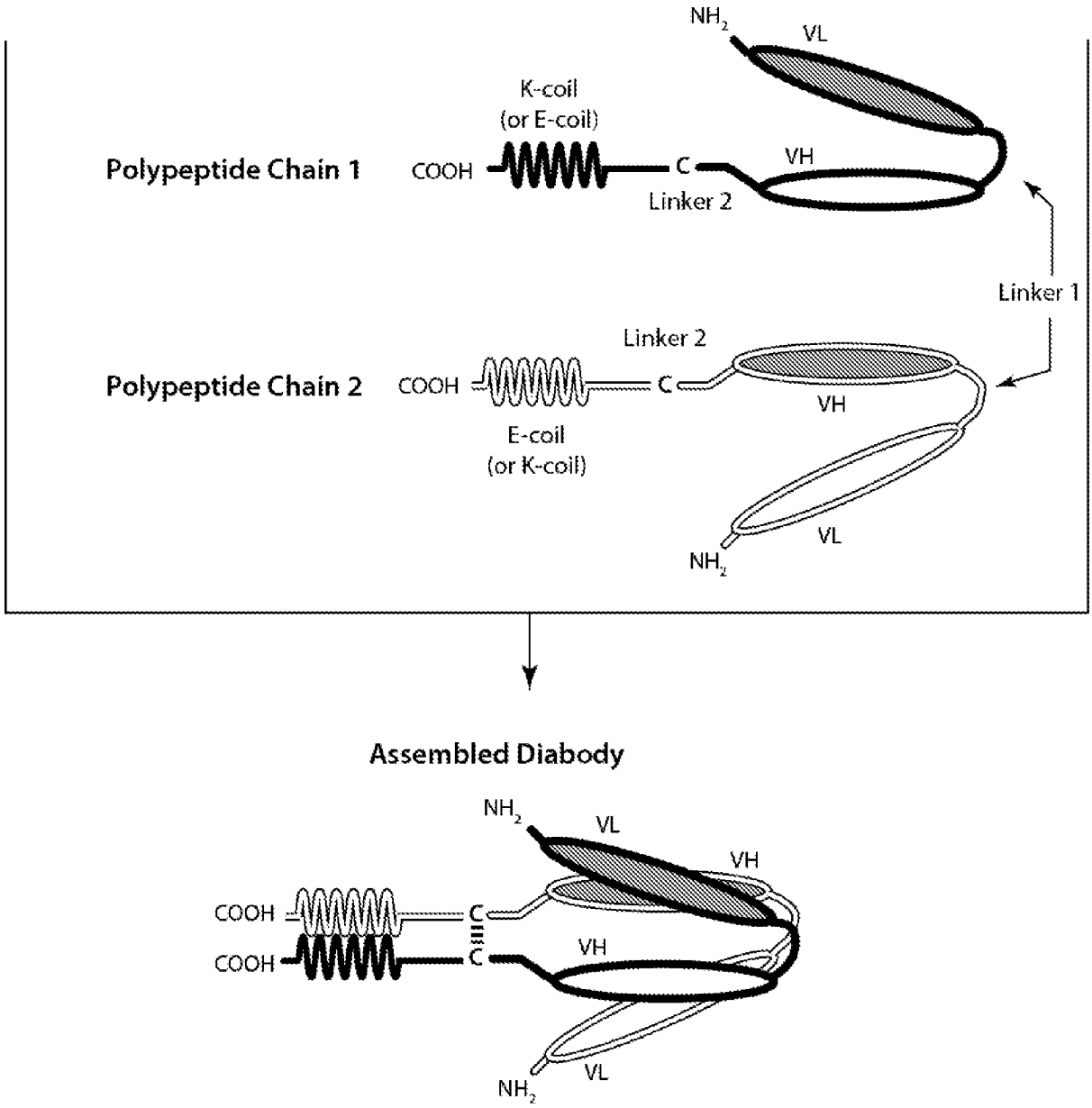
NH2
VL
K-coil
(or E-coil)
Polypeptide Chain 1
COOH
C
VH
Linker 2
Linker 1
Linker 2
Polypeptide Chain 2
COOH
C
VH
E-coil
(or K-coil)
VL
NH2
Assembled Diabody
NH2
VL
VH
COOH
C
COOH
C
VH
VL
NH2

Figure 1

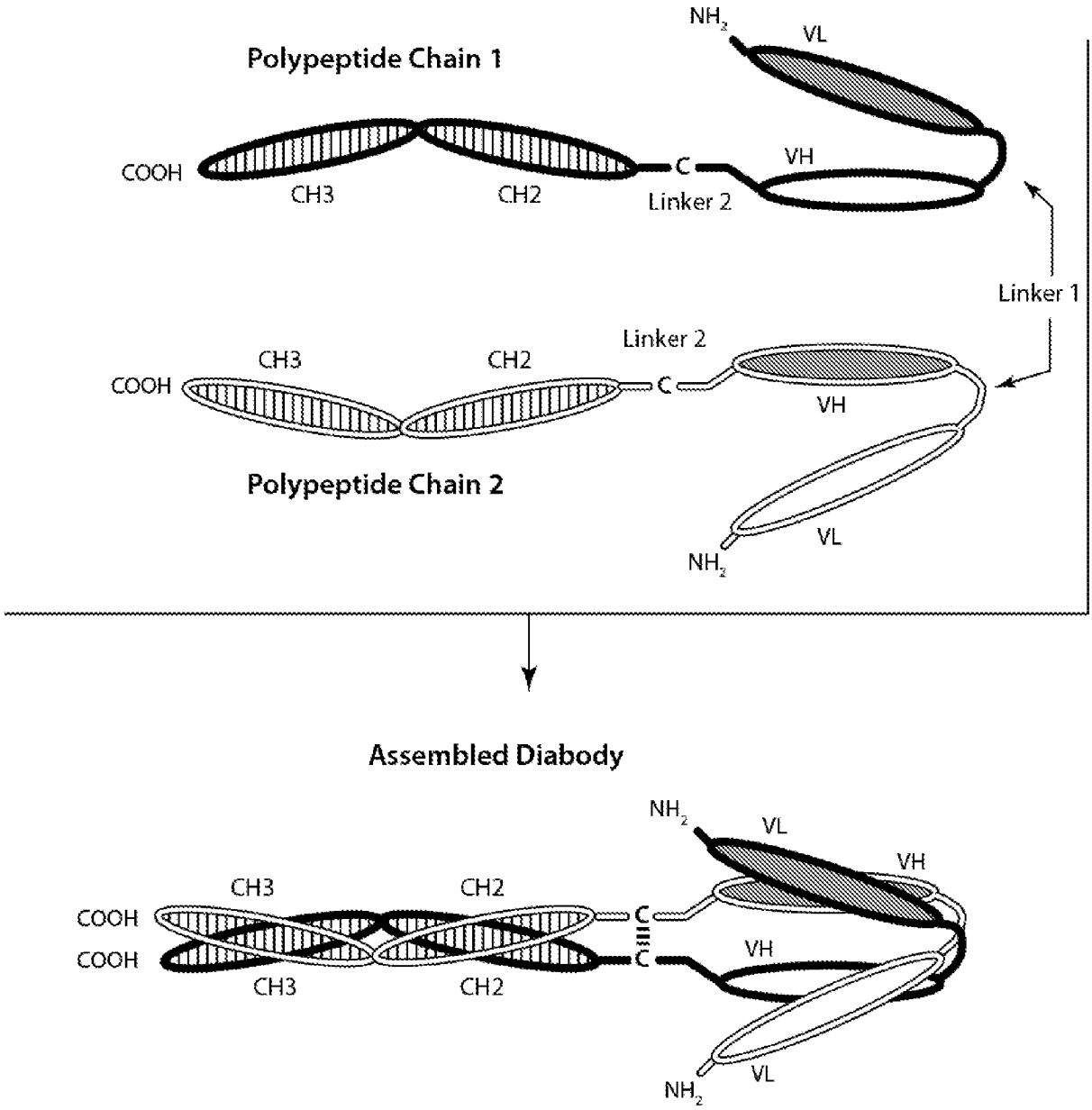
Polypeptide Chain 1
NH2
VL
COOH
CH3
CH2
C
Linker 2
VH
Linker 1
CH3
CH2
Linker 2
COOH
C
VH
Polypeptide Chain 2
VL
NH2
Assembled Diabody
NH2
VL
VH
CH3
CH2
COOH
C
COOH
C
VH
CH3
CH2
VL
NH2

Figure 2

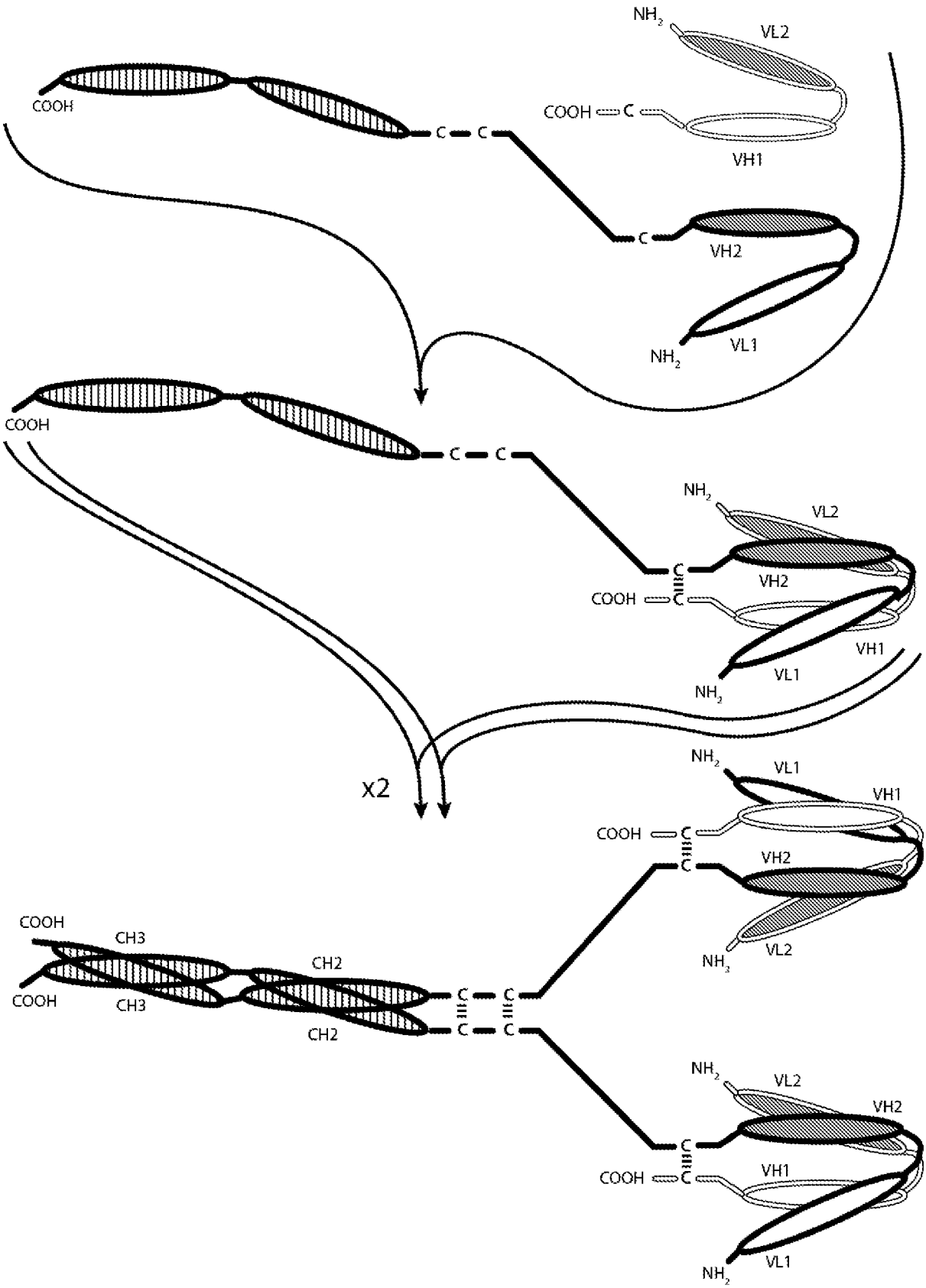
NH2
VL2
COOH
C
C
COOH
C
VH1
C
VH2
NH2
VL1
COOH
C
C
NH2
VL2
C
VH2
COOH
C
VH1
NH2
VL1
x2
NH2
VL1
VH1
COOH
C
VH2
C
NH2
VL2
COOH
CH3
CH2
COOH
CH3
CH2
C
C
C
C
NH2
VL2
VH2
C
COOH
C
VH1
NH2
VL1

Figure 3A

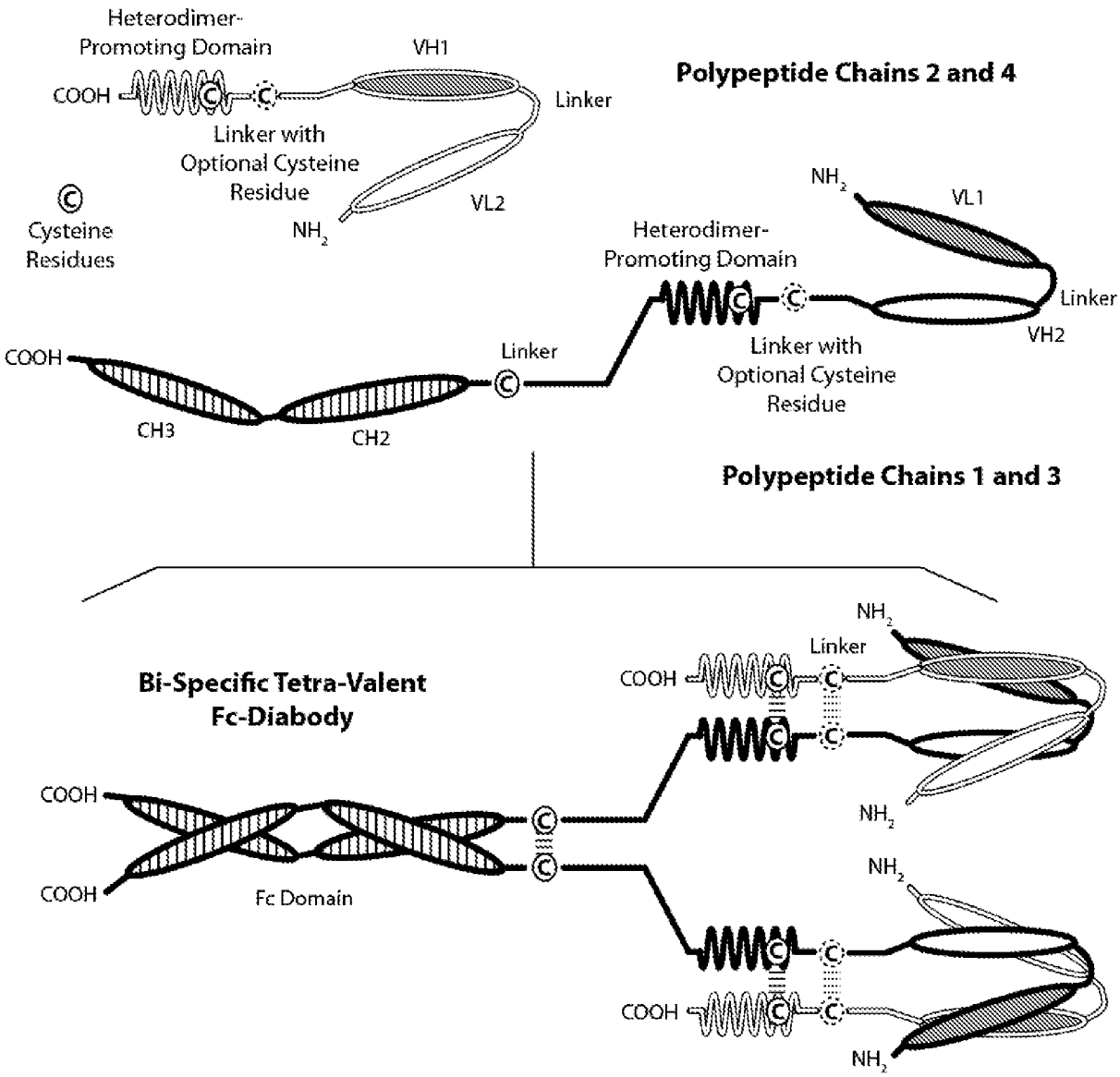
Heterodimer-
Promoting Domain
VH1
COOH
Linker
Linker with
Optional Cysteine
Residue
VL2
NH2
Cysteine
Residues
Polypeptide Chains 2 and 4
NH2
VL1
Heterodimer-
Promoting Domain
Linker
VH2
Linker with
Optional Cysteine
Residue
COOH
Linker
CH3
CH2
Polypeptide Chains 1 and 3
Bi-Specific Tetra-Valent
Fc-Diabody
NH2
Linker
COOH
COOH
COOH
Fc Domain
NH2
NH2
COOH
NH2

Figure 3B

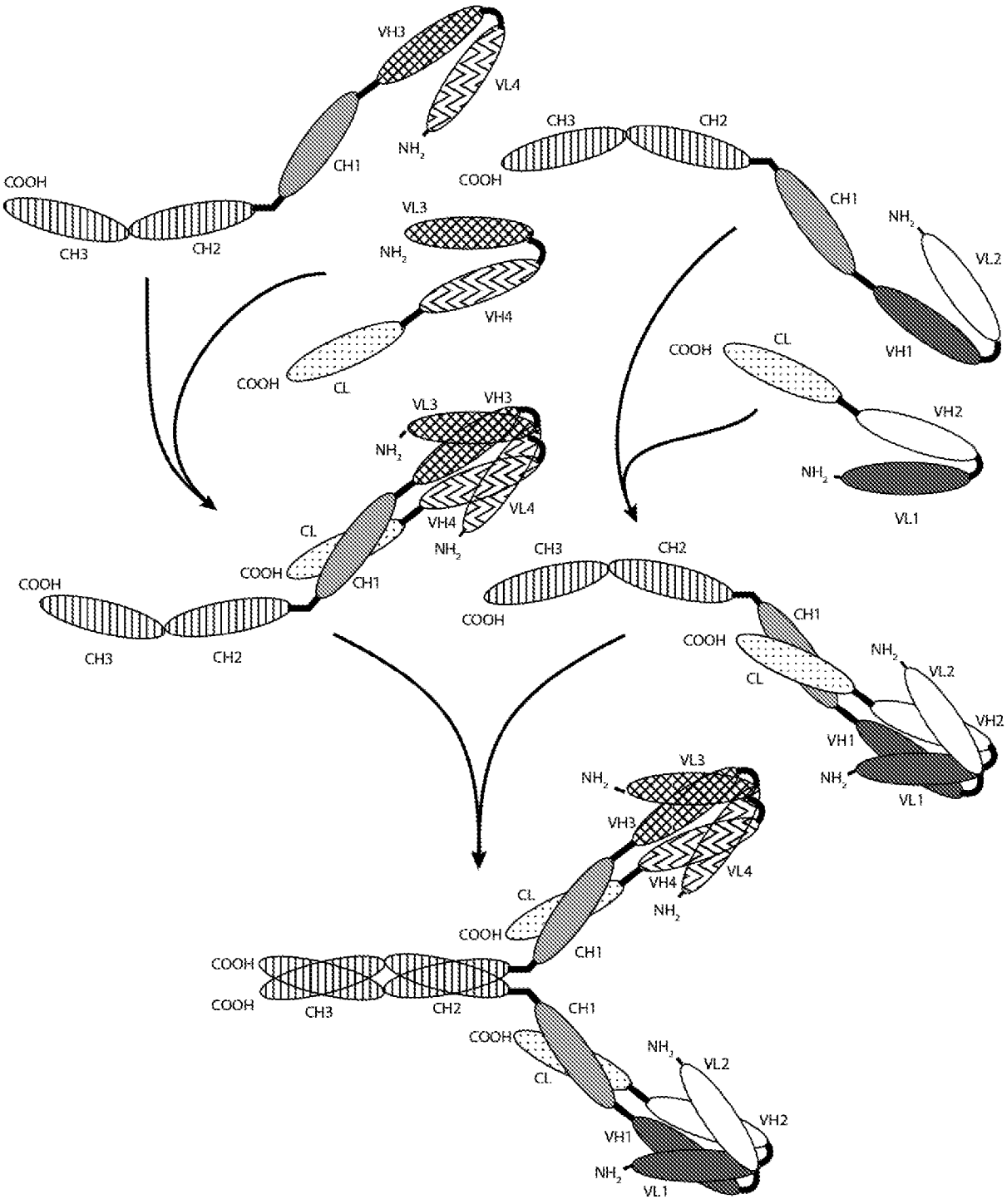

Figure 3C

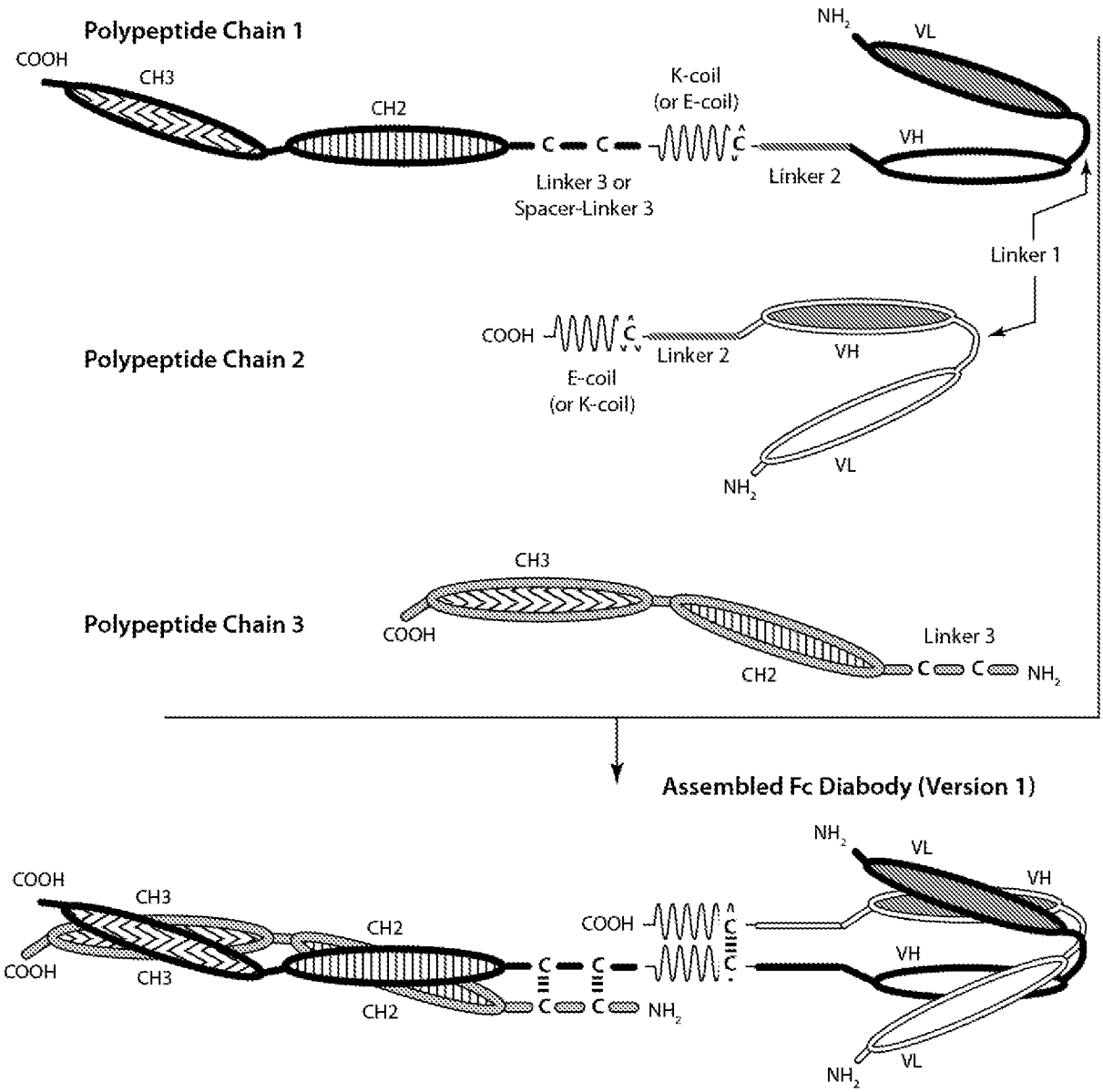
Polypeptide Chain 1
COOH
CH3
CH2
K-coil
(or E-coil)
NH2
VL
VH
Linker 3 or
Spacer-Linker 3
Linker 2
Linker 1
Polypeptide Chain 2
COOH
E-coil
(or K-coil)
Linker 2
VH
VL
NH2
Polypeptide Chain 3
CH3
COOH
Linker 3
CH2
NH2
Assembled Fc Diabody (Version 1)
COOH
CH3
CH3
CH2
CH2
NH2
COOH
NH2
VL
VH
VH
VL
NH2

Figure 4A

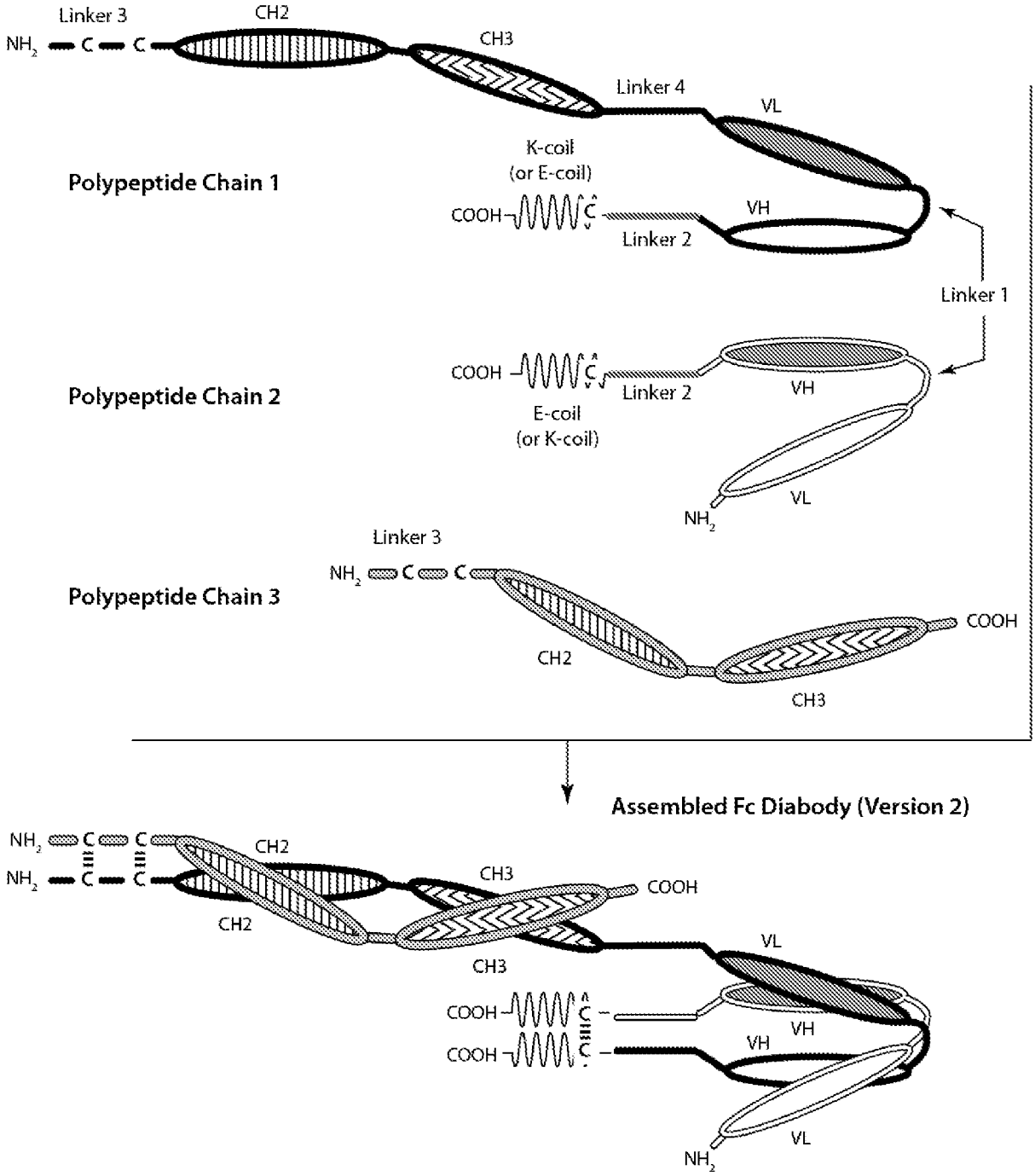
Linker 3
CH2
CH3
Linker 4
VL
K-coil
(or E-coil)
Polypeptide Chain 1
COOH
VH
Linker 2
Linker 1
Polypeptide Chain 2
COOH
Linker 2
VH
E-coil
(or K-coil)
VL
Linker 3
Polypeptide Chain 3
CH2
COOH
CH3
Assembled Fc Diabody (Version 2)
CH2
CH3
COOH
CH2
CH3
VL
COOH
VH
COOH
VH
VL

Figure 4B

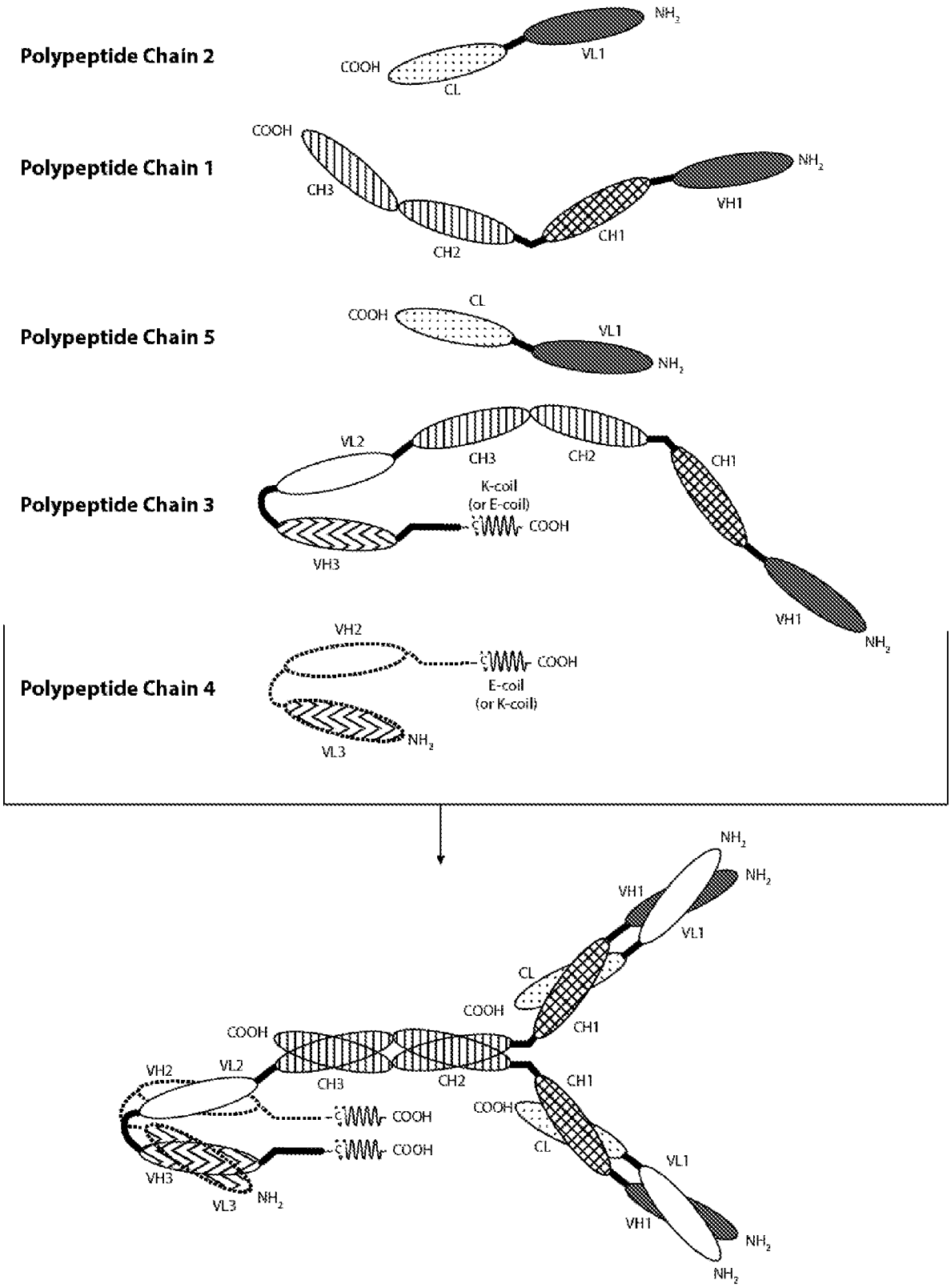
Polypeptide Chain 2
NH2
VL1
COOH
CL
Polypeptide Chain 1
COOH
CH3
CH2
CH1
VH1
NH2
Polypeptide Chain 5
CL
COOH
VL1
NH2
Polypeptide Chain 3
VL2
CH3
CH2
CH1
K-coil
(or E-coil)
COOH
VH3
VH1
NH2
Polypeptide Chain 4
VH2
COOH
E-coil
(or K-coil)
VL3
NH2
NH2
NH2
VH1
VL1
CL
COOH
CH1
COOH
VH2
VL2
CH3
CH2
CH1
COOH
COOH
CL
VL1
VH3
VL3
NH2
VH1
NH2
NH2

Figure 5

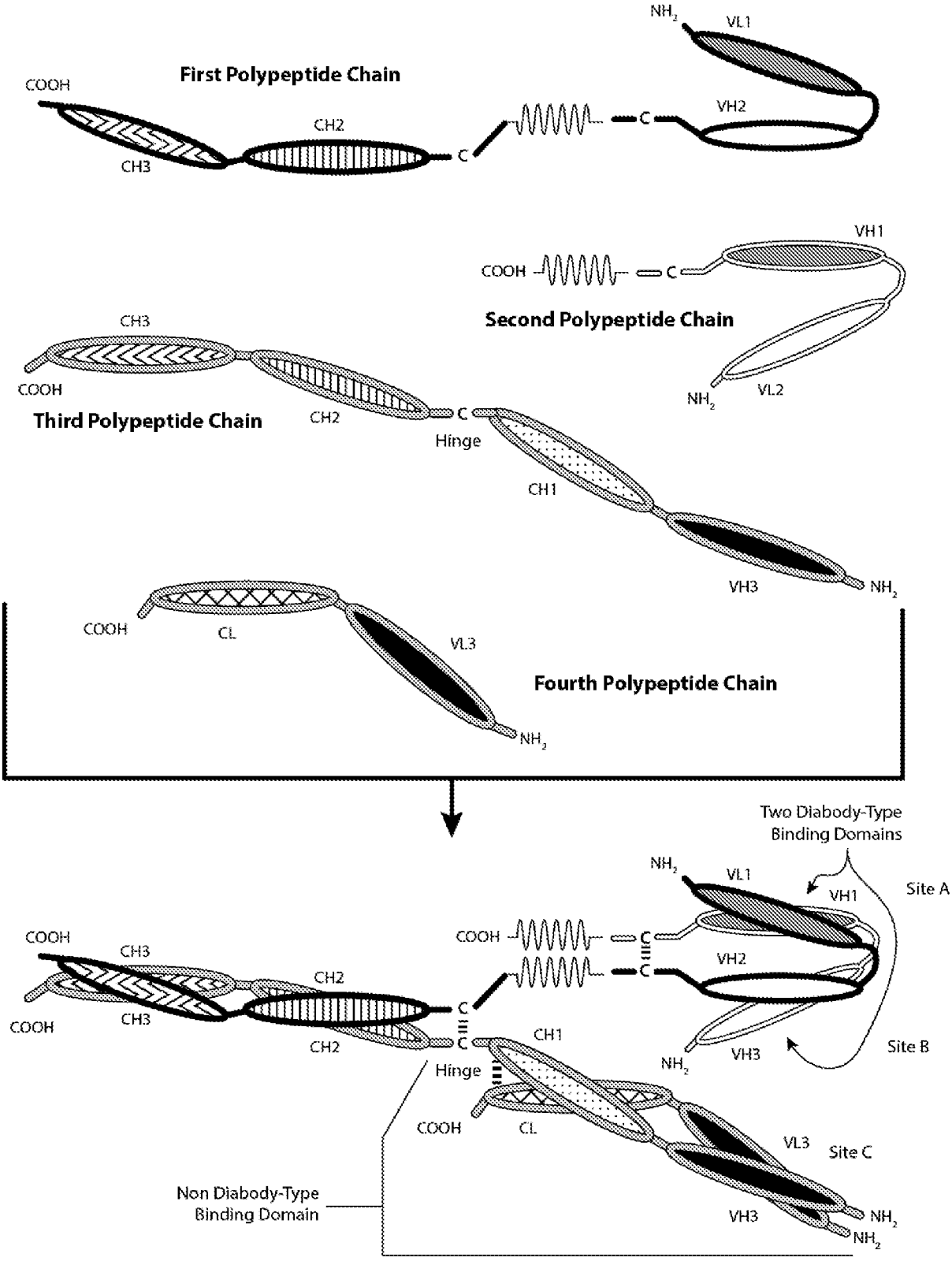
First Polypeptide Chain
COOH
CH2
CH3
C
NH2
VL1
VH2
Second Polypeptide Chain
COOH
C
VH1
NH2
VL2
Third Polypeptide Chain
CH3
COOH
CH2
C
Hinge
CH1
VH3
NH2
Fourth Polypeptide Chain
COOH
CL
VL3
NH2
Two Diabody-Type Binding Domains
Site A
Site B
Site C
Non Diabody-Type Binding Domain

Figure 6A

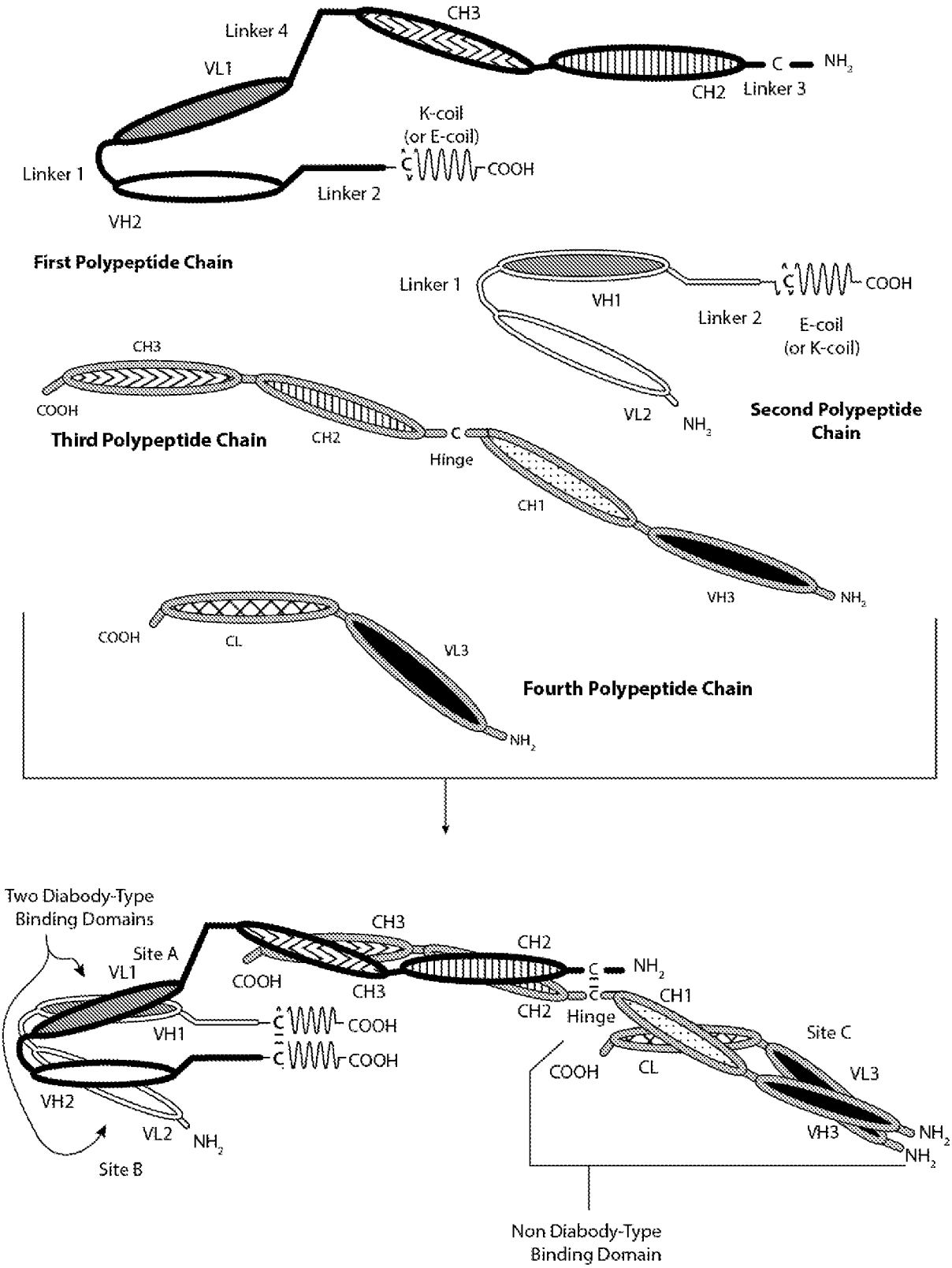
CH3
Linker 4
VL1
C
NH2
CH2
Linker 3
K-coil
(or E-coil)
Linker 1
COOH
Linker 2
VH2
First Polypeptide Chain
Linker 1
VH1
COOH
Linker 2
E-coil
(or K-coil)
CH3
VL2
NH2
Second Polypeptide Chain
COOH
Third Polypeptide Chain
CH2
C
Hinge
CH1
VH3
NH2
COOH
CL
VL3
Fourth Polypeptide Chain
NH2
Two Diabody-Type
Binding Domains
Site A
VL1
CH3
CH2
COOH
C
NH2
CH3
CH2
CH1
VH1
COOH
Hinge
Site C
COOH
CL
VL3
VH2
VH3
NH2
VL2
NH2
NH2
Site B
Non Diabody-Type
Binding Domain

Figure 6B

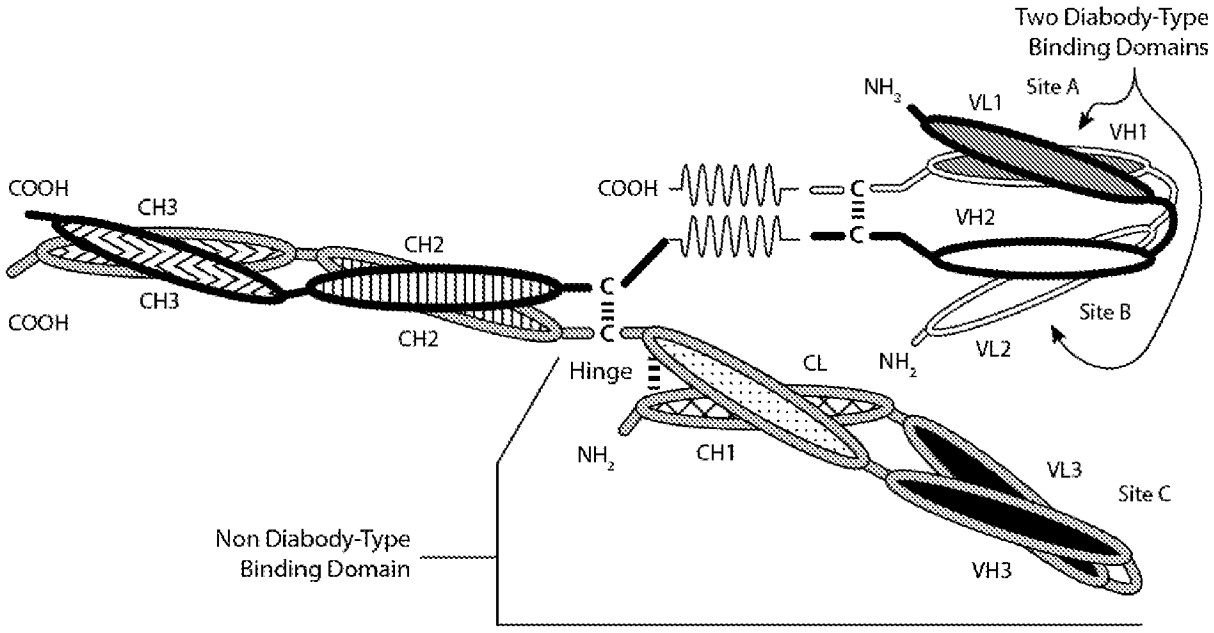

Figure 6C

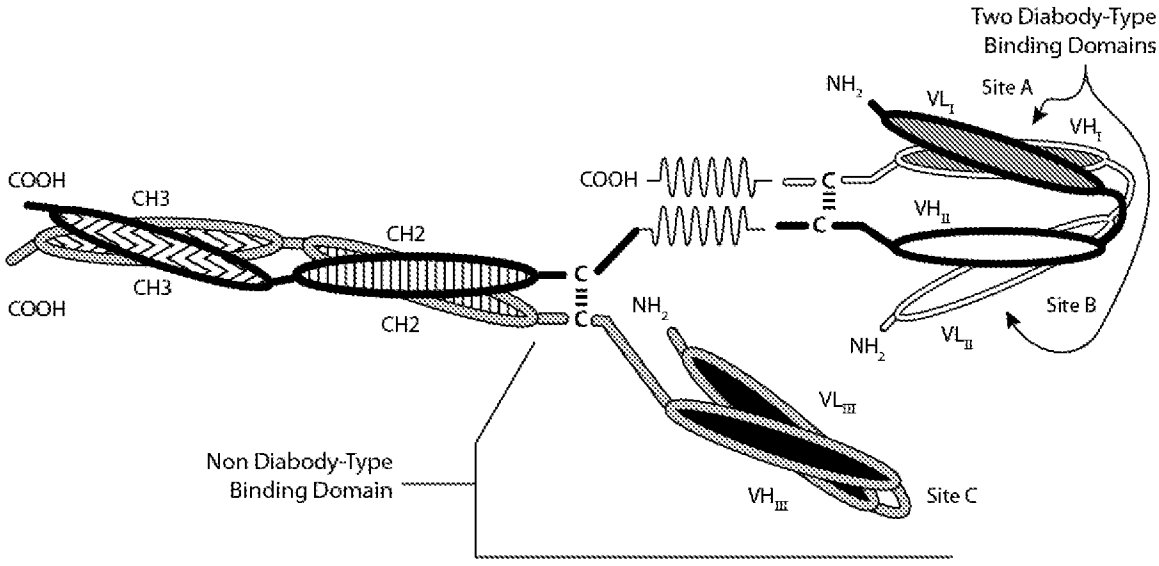

Figure 6D

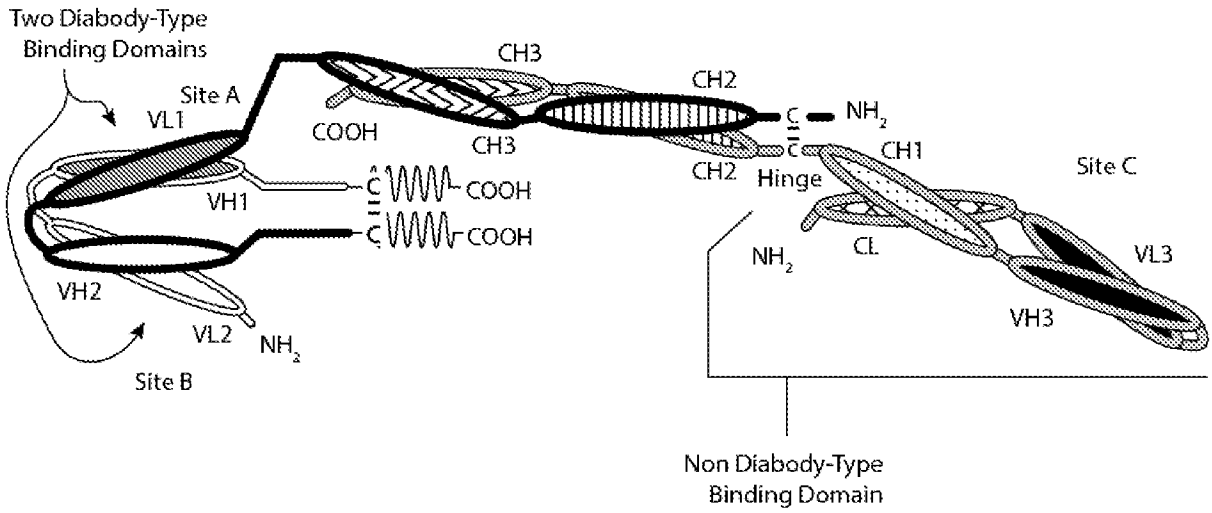

Figure 6E

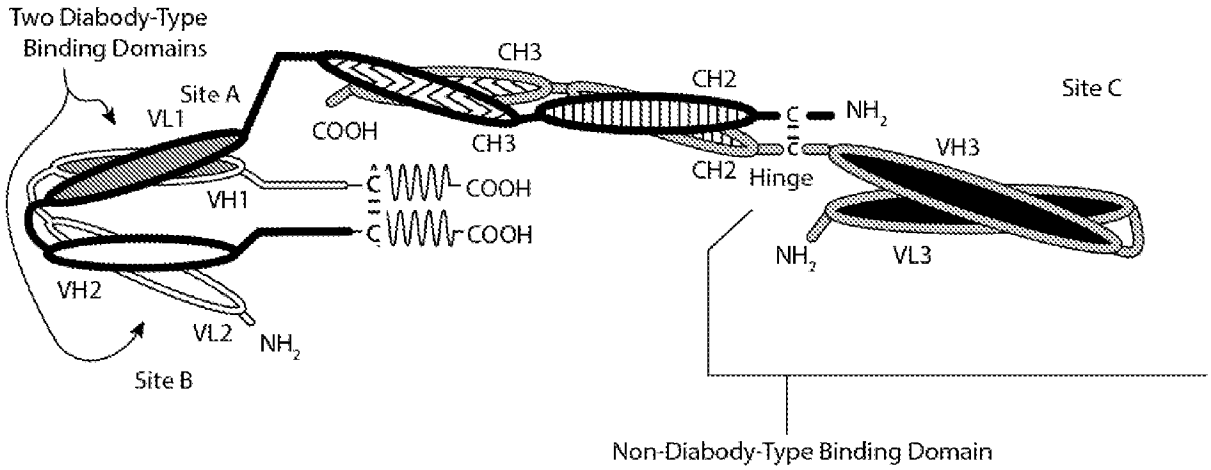

Figure 6F

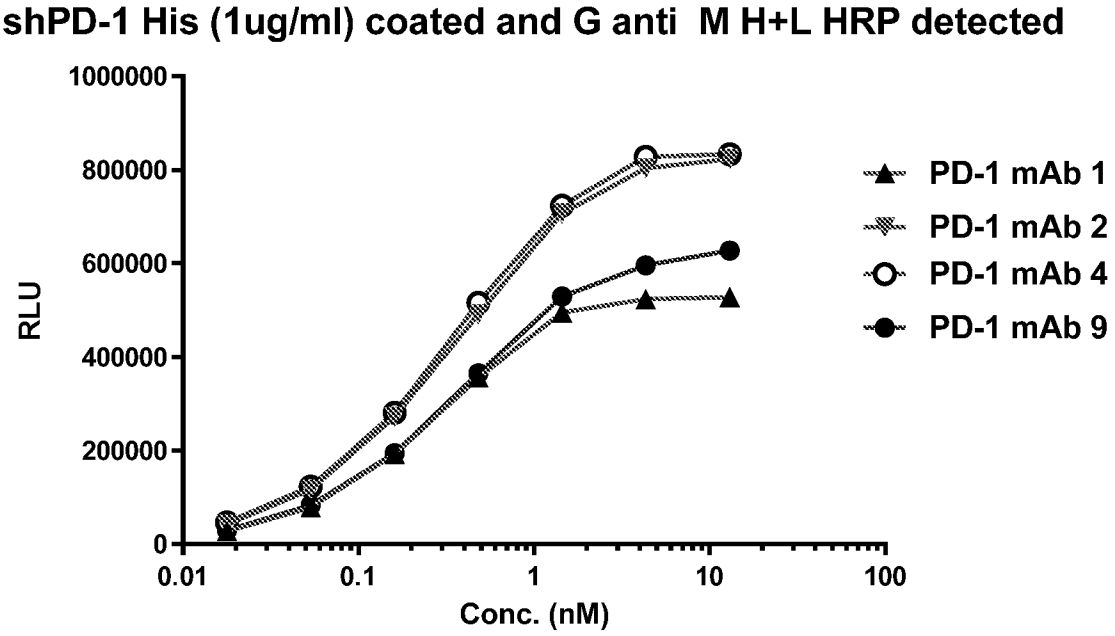

Figure 7A

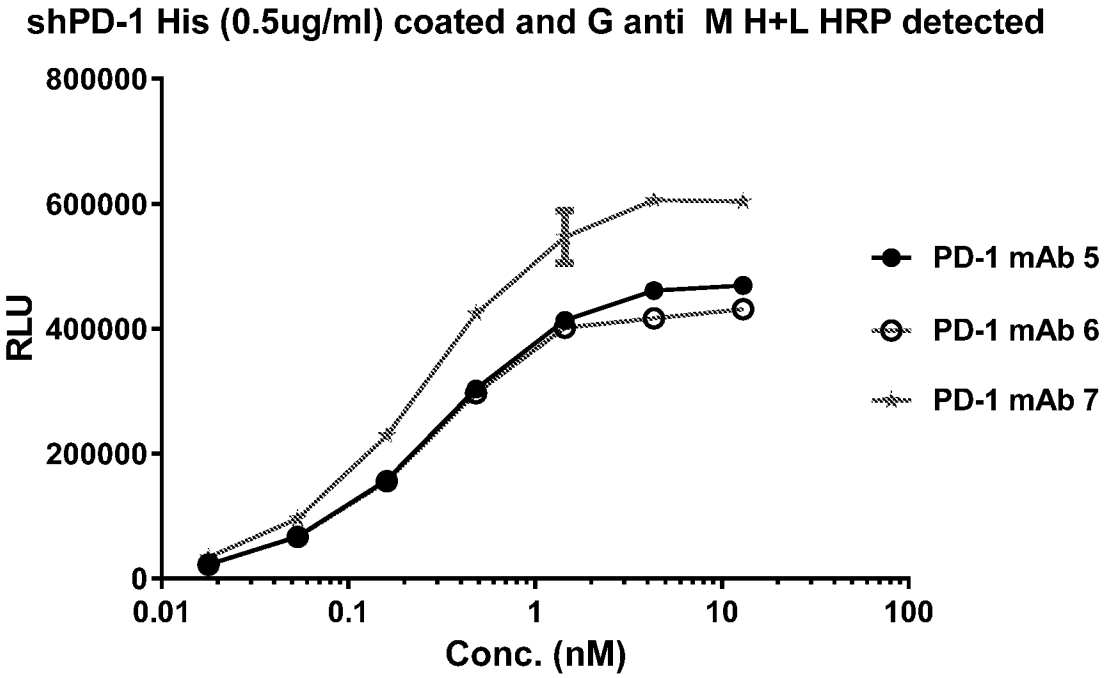

Figure 7B

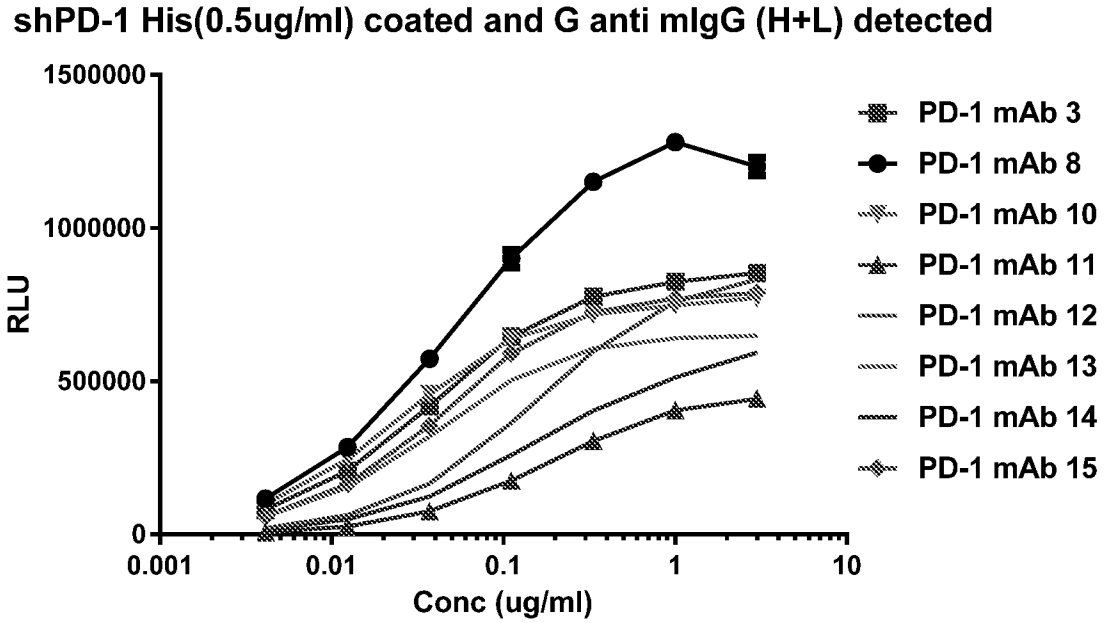

Figure 7C

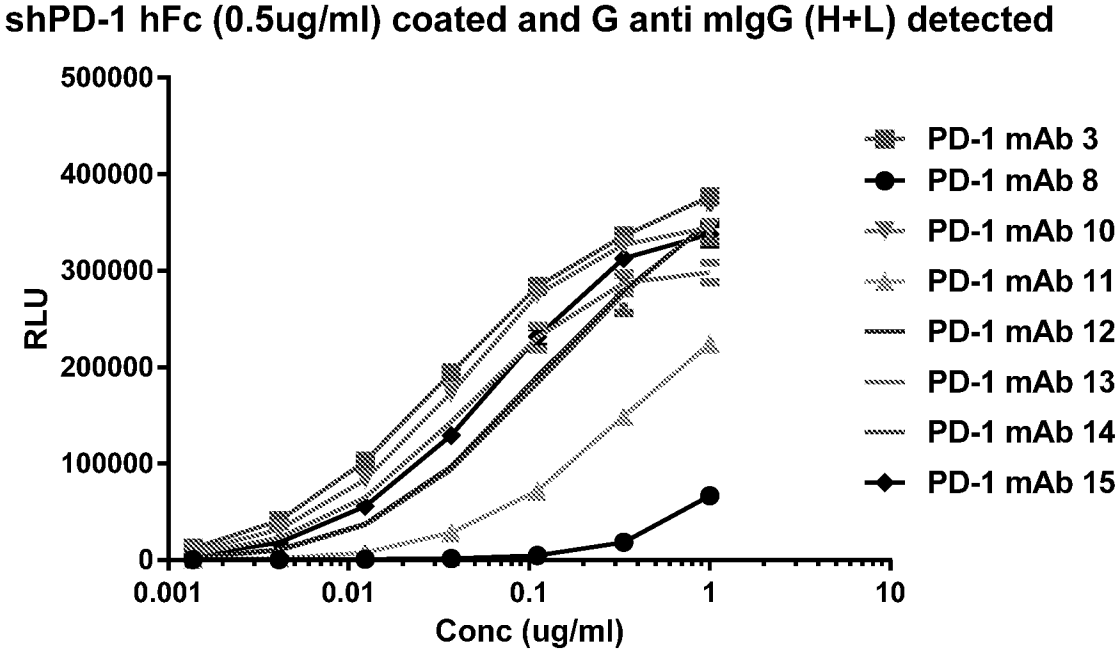

Figure 7D

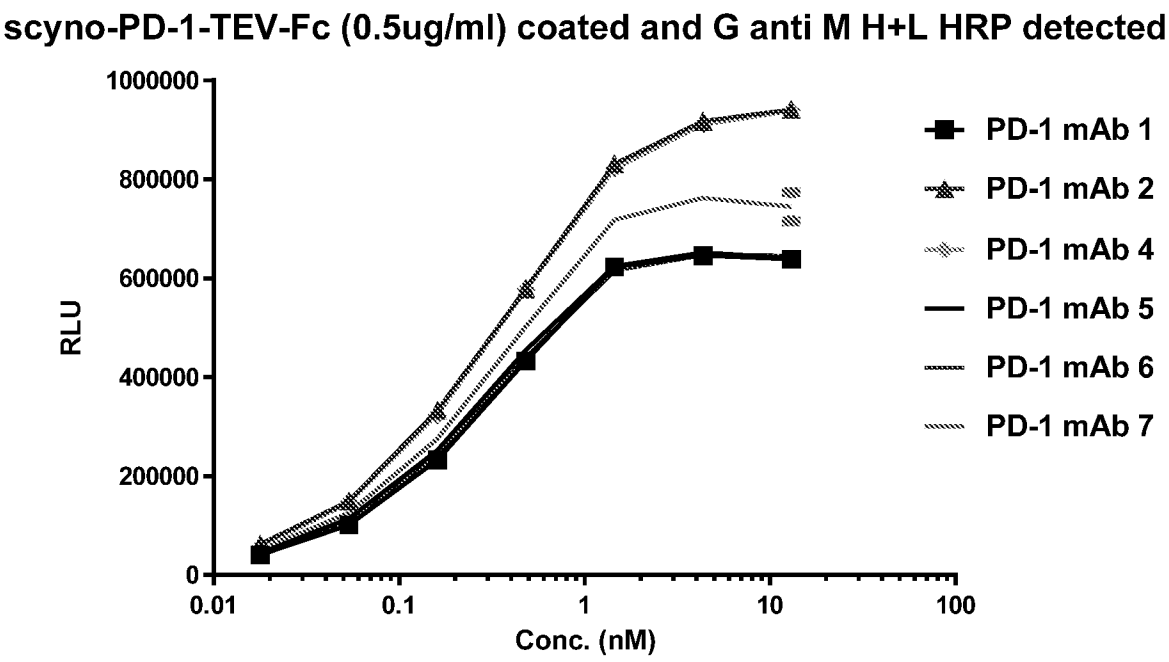

Figure 8A

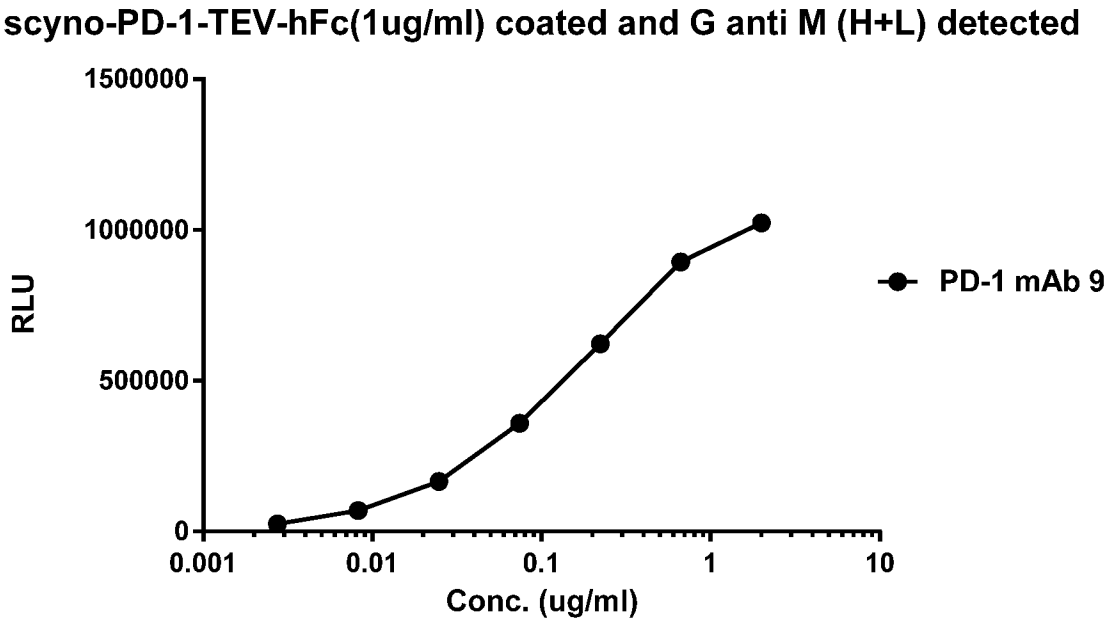

Figure 8B

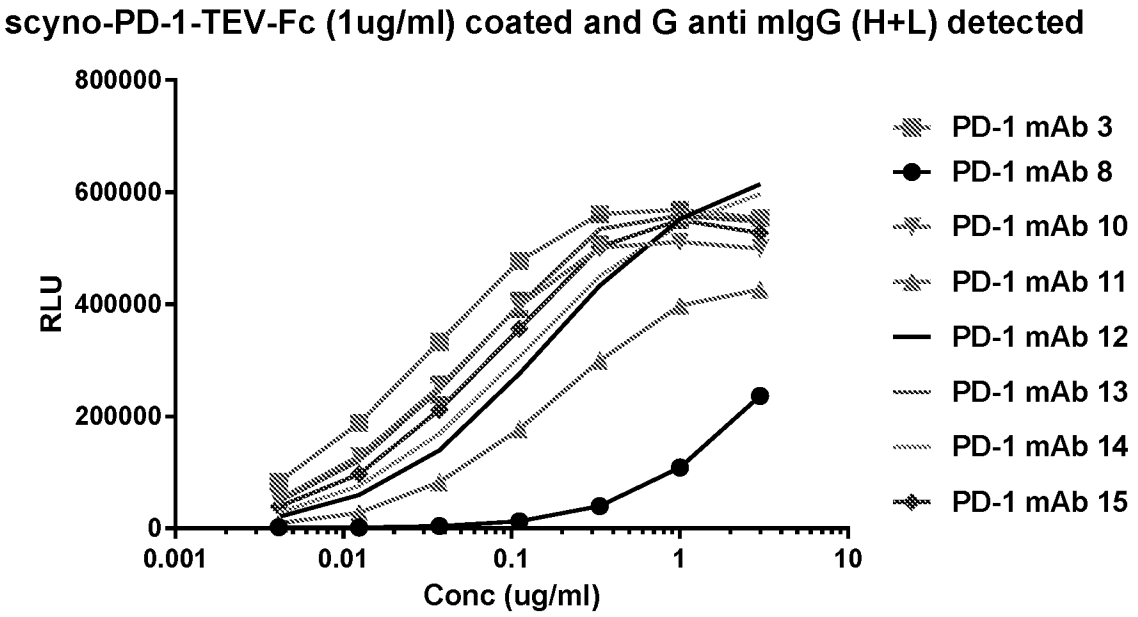
scyno-PD-1-TEV-Fc (1ug/ml) coated and G anti mIgG (H+L) detected
800000
600000
400000
200000
0
RLU
0.001
0.01
0.1
1
10
Conc (ug/ml)
PD-1 mAb 3
PD-1 mAb 8
PD-1 mAb 10
PD-1 mAb 11
PD-1 mAb 12
PD-1 mAb 13
PD-1 mAb 14
PD-1 mAb 15

Figure 8C

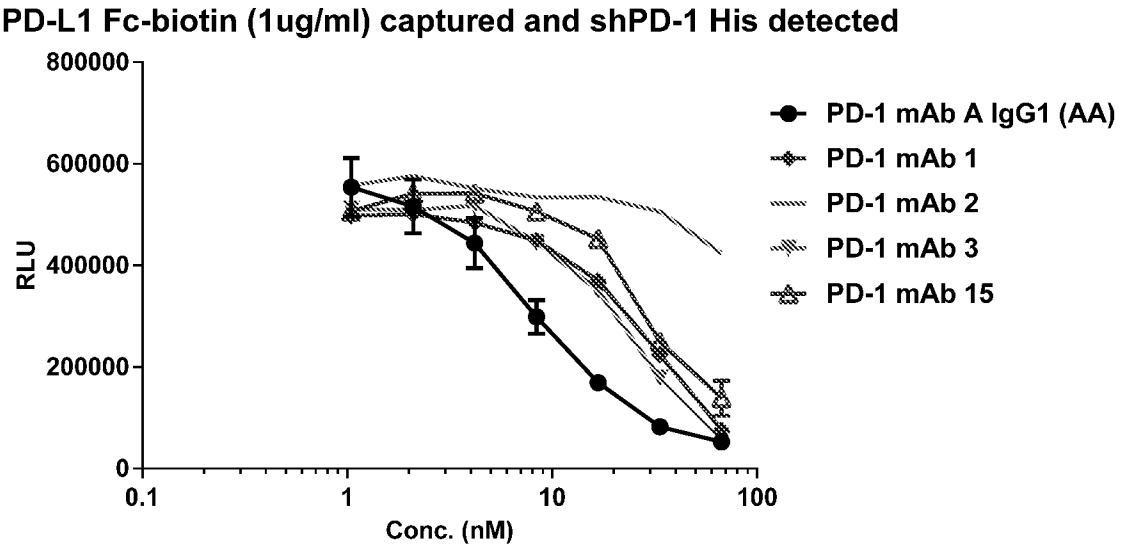

Figure 9A

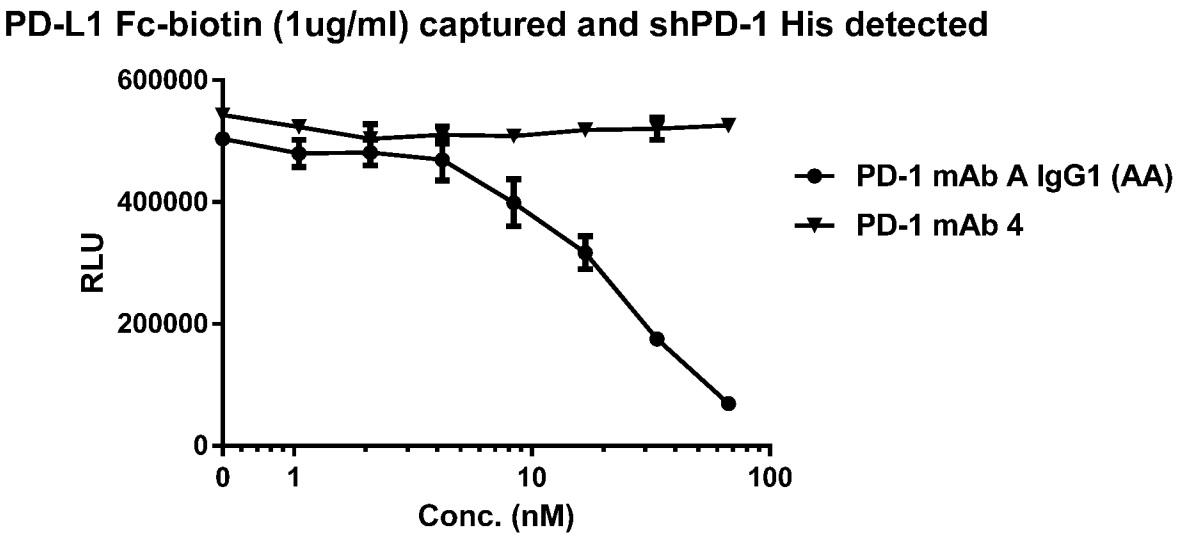

Figure 9B

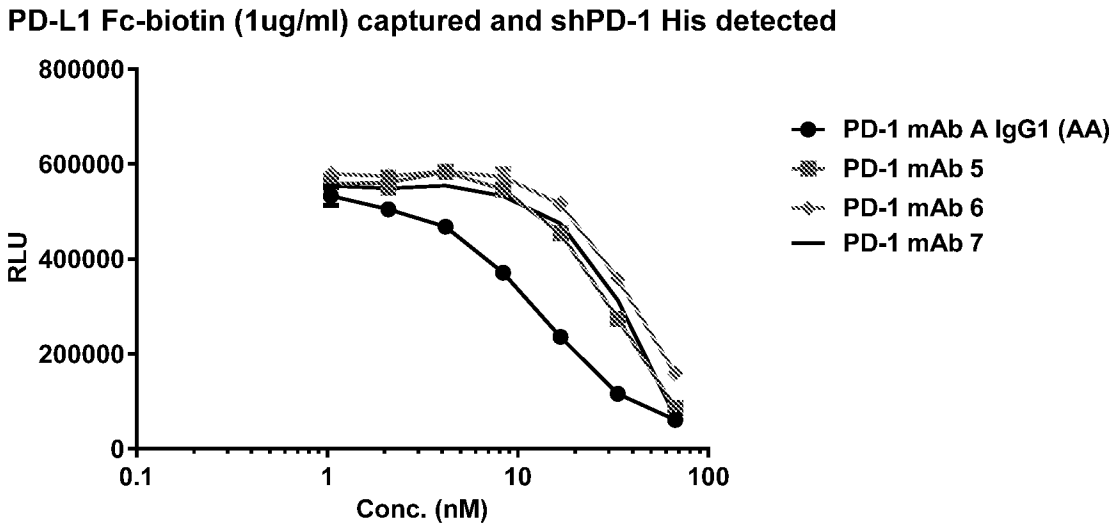

Figure 9C

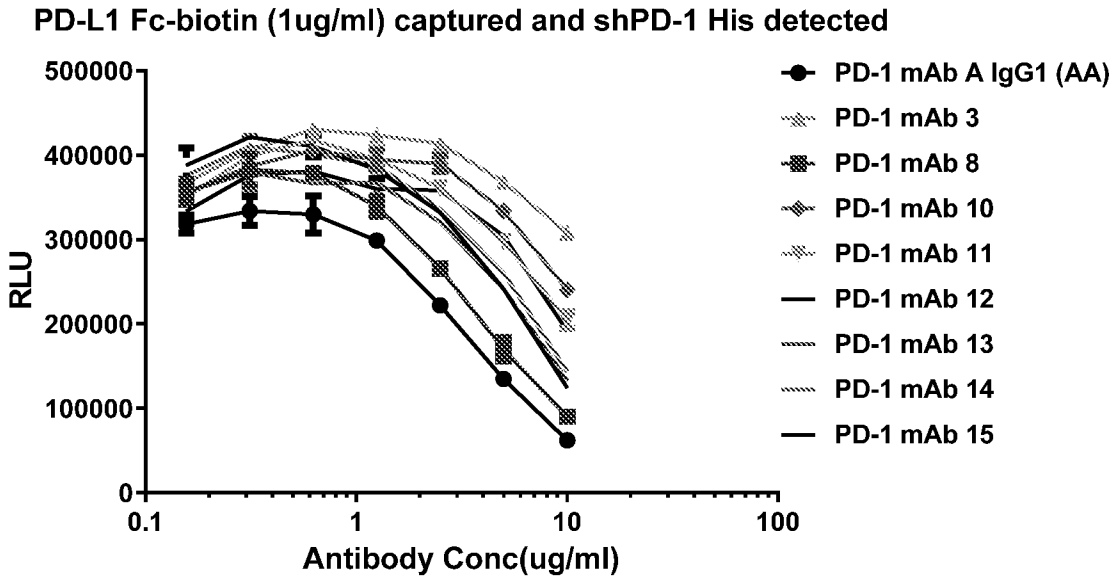

Figure 9D

Normal Tissues

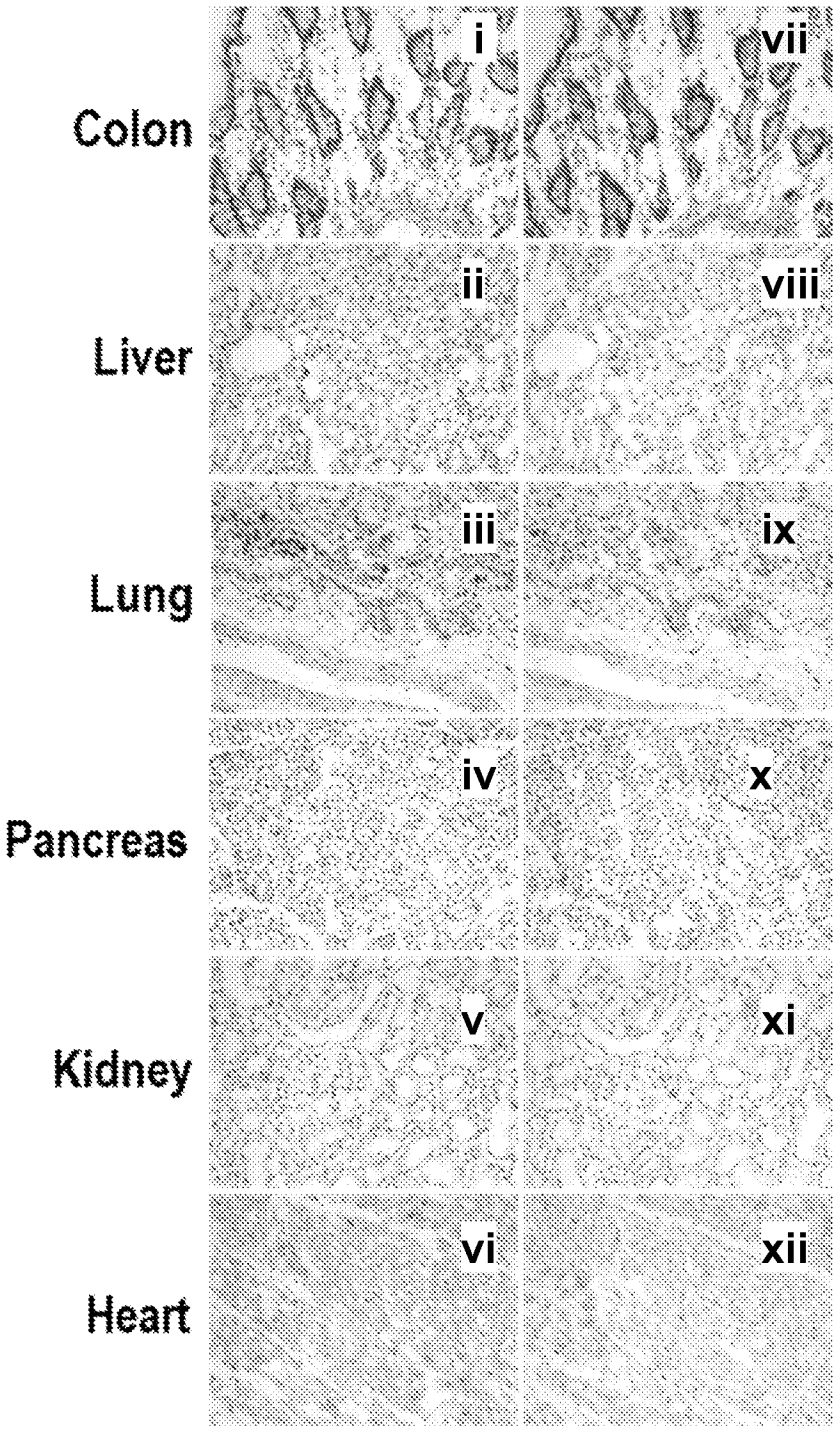

Figure 10A

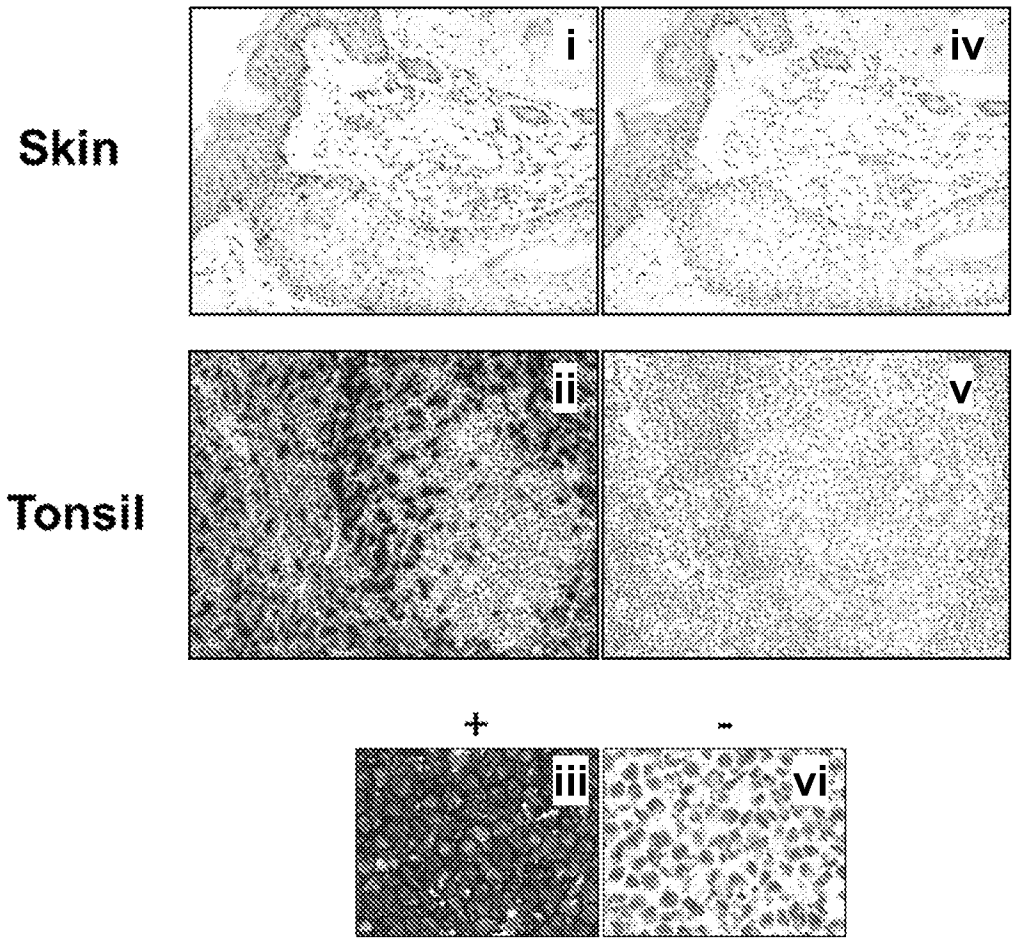
Skin
Tonsil
i
iv
ii
v
+
-
iii
vi
PD-1 mAb 7
0.313 µg/ml
Isotype Control
0.313 µg/ml

Figure 10B

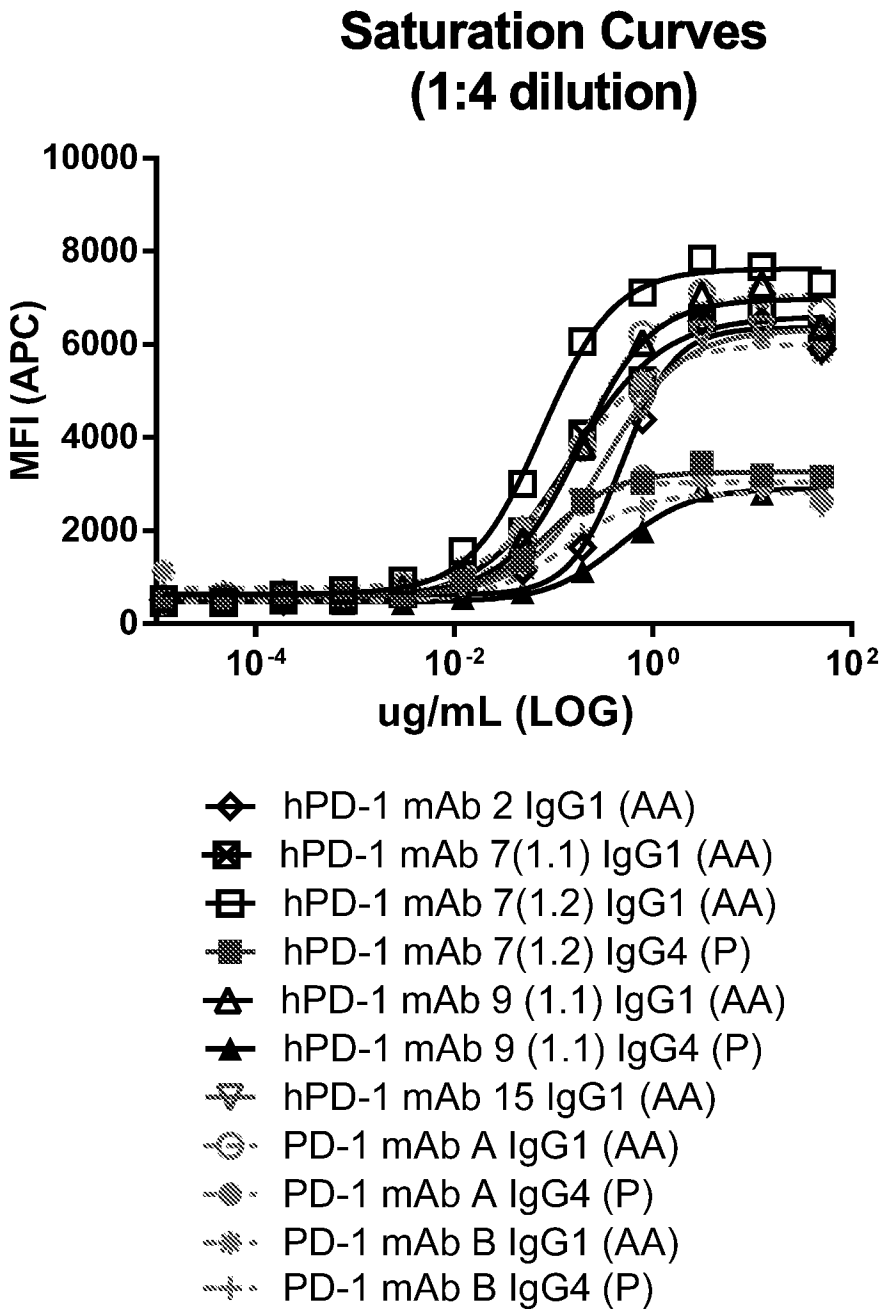
Saturation Curves
(1:4 dilution)
10000
8000
6000
4000
2000
0
MFI (APC)
10-4
10-2
100
102
ug/mL (LOG)
hPD-1 mAb 2 IgG1 (AA)
hPD-1 mAb 7(1.1) IgG1 (AA)
hPD-1 mAb 7(1.2) IgG1 (AA)
hPD-1 mAb 7(1.2) IgG4 (P)
hPD-1 mAb 9 (1.1) IgG1 (AA)
hPD-1 mAb 9 (1.1) IgG4 (P)
hPD-1 mAb 15 IgG1 (AA)
PD-1 mAb A IgG1 (AA)
PD-1 mAb A IgG4 (P)
PD-1 mAb B IgG1 (AA)
PD-1 mAb B IgG4 (P)

Figure 11

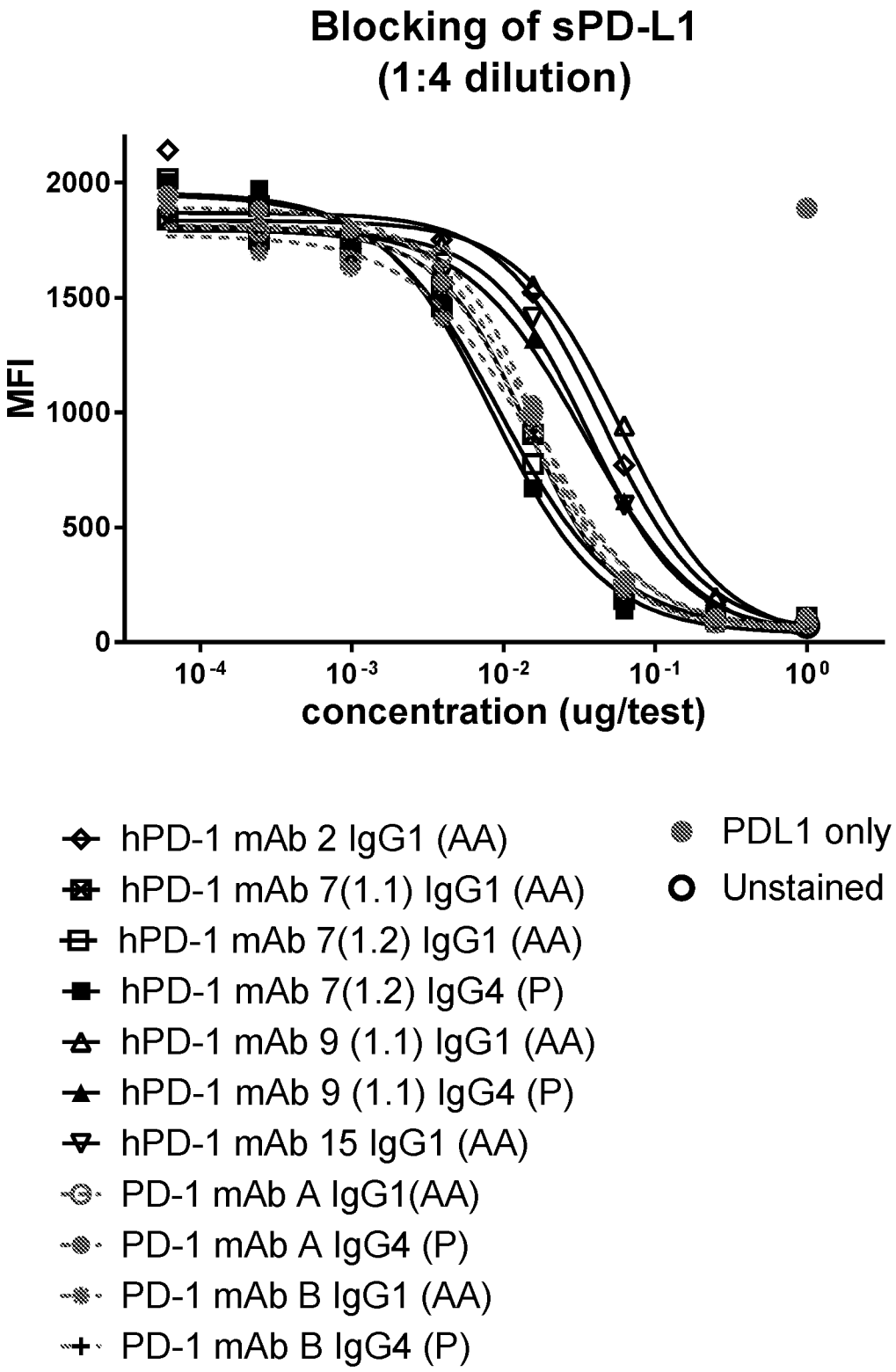
Blocking of sPD-L1
(1:4 dilution)
MFI
2000
1500
1000
500
0
10-4
10-3
10-2
10-1
100
concentration (ug/test)
hPD-1 mAb 2 IgG1 (AA)
hPD-1 mAb 7(1.1) IgG1 (AA)
hPD-1 mAb 7(1.2) IgG1 (AA)
hPD-1 mAb 7(1.2) IgG4 (P)
hPD-1 mAb 9 (1.1) IgG1 (AA)
hPD-1 mAb 9 (1.1) IgG4 (P)
hPD-1 mAb 15 IgG1 (AA)
PD-1 mAb A IgG1(AA)
PD-1 mAb A IgG4 (P)
PD-1 mAb B IgG1 (AA)
PD-1 mAb B IgG4 (P)
PDL1 only
Unstained

Figure 12A

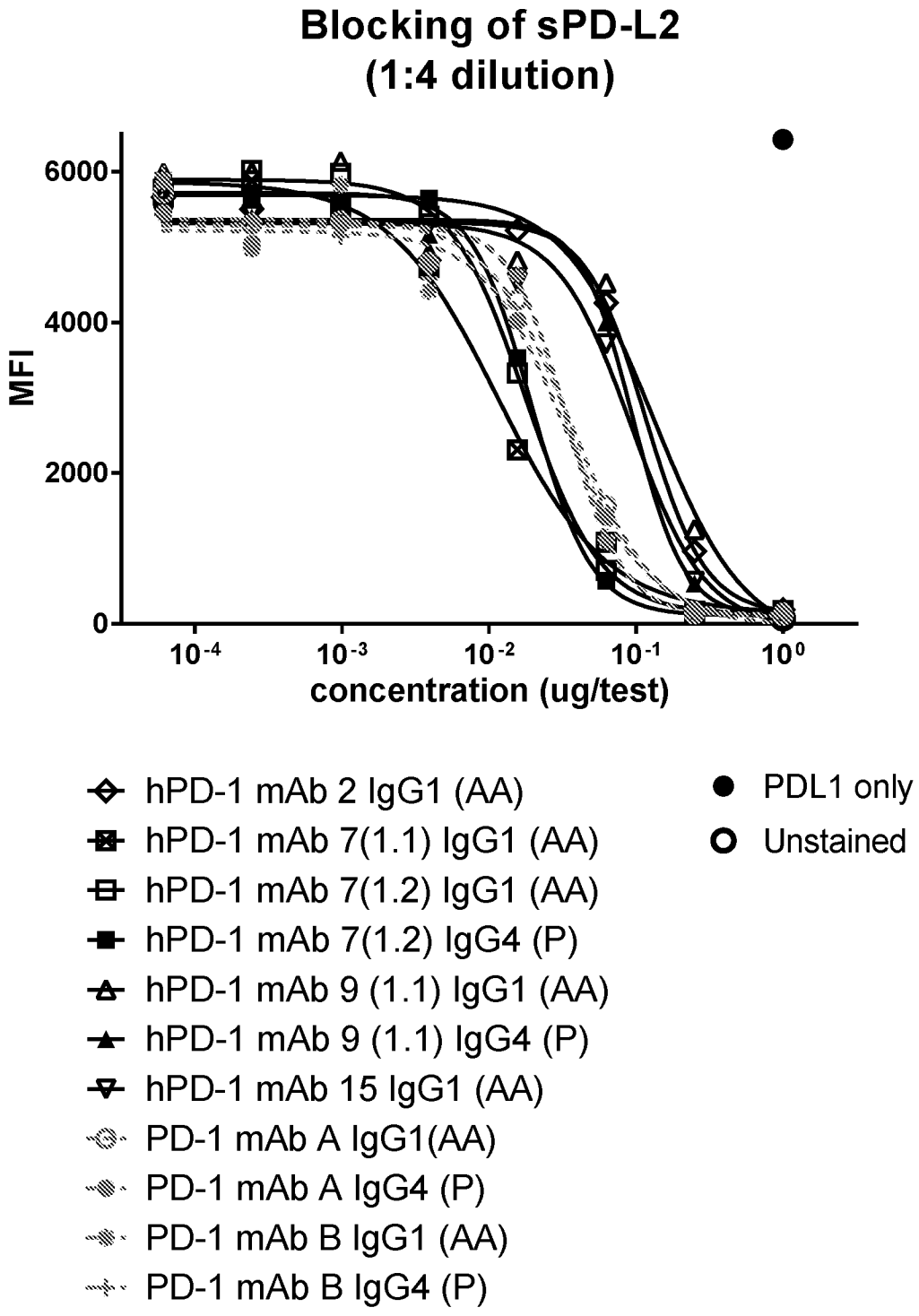
Blocking of sPD-L2
(1:4 dilution)
6000
4000
2000
0
MFI
10-4
10-3
10-2
10-1
100
concentration (ug/test)
hPD-1 mAb 2 IgG1 (AA)
hPD-1 mAb 7(1.1) IgG1 (AA)
hPD-1 mAb 7(1.2) IgG1 (AA)
hPD-1 mAb 7(1.2) IgG4 (P)
hPD-1 mAb 9 (1.1) IgG1 (AA)
hPD-1 mAb 9 (1.1) IgG4 (P)
hPD-1 mAb 15 IgG1 (AA)
PD-1 mAb A IgG1(AA)
PD-1 mAb A IgG4 (P)
PD-1 mAb B IgG1 (AA)
PD-1 mAb B IgG4 (P)
PDL1 only
Unstained

Figure 12B

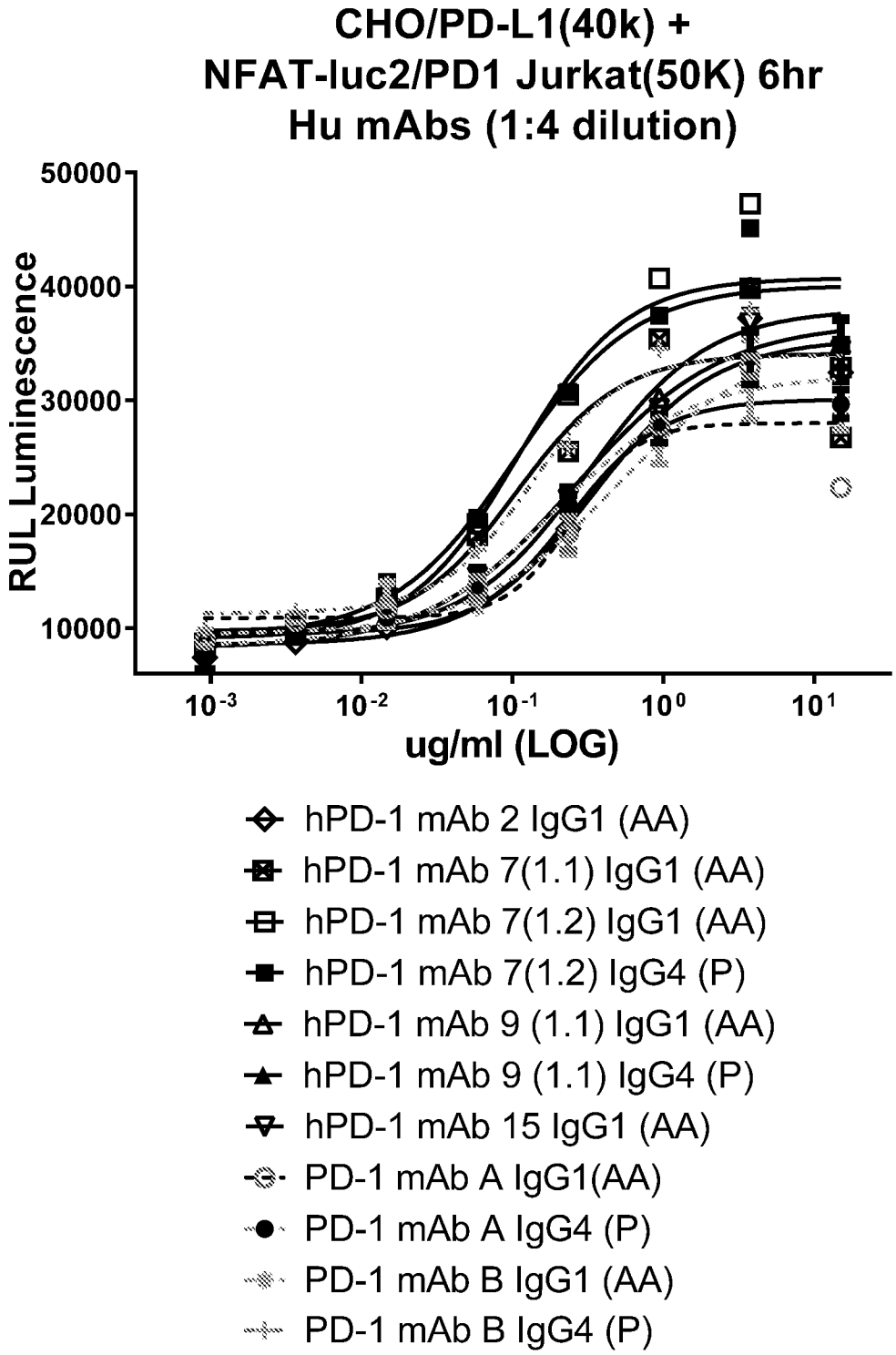
CHO/PD-L1(40k) +
NFAT-luc2/PD1 Jurkat(50K) 6hr
Hu mAbs (1:4 dilution)
RUL Luminescence
50000
40000
30000
20000
10000
10-3
10-2
10-1
100
101
ug/ml (LOG)
hPD-1 mAb 2 IgG1 (AA)
hPD-1 mAb 7(1.1) IgG1 (AA)
hPD-1 mAb 7(1.2) IgG1 (AA)
hPD-1 mAb 7(1.2) IgG4 (P)
hPD-1 mAb 9 (1.1) IgG1 (AA)
hPD-1 mAb 9 (1.1) IgG4 (P)
hPD-1 mAb 15 IgG1 (AA)
PD-1 mAb A IgG1(AA)
PD-1 mAb A IgG4 (P)
PD-1 mAb B IgG1 (AA)
PD-1 mAb B IgG4 (P)

Figure 13

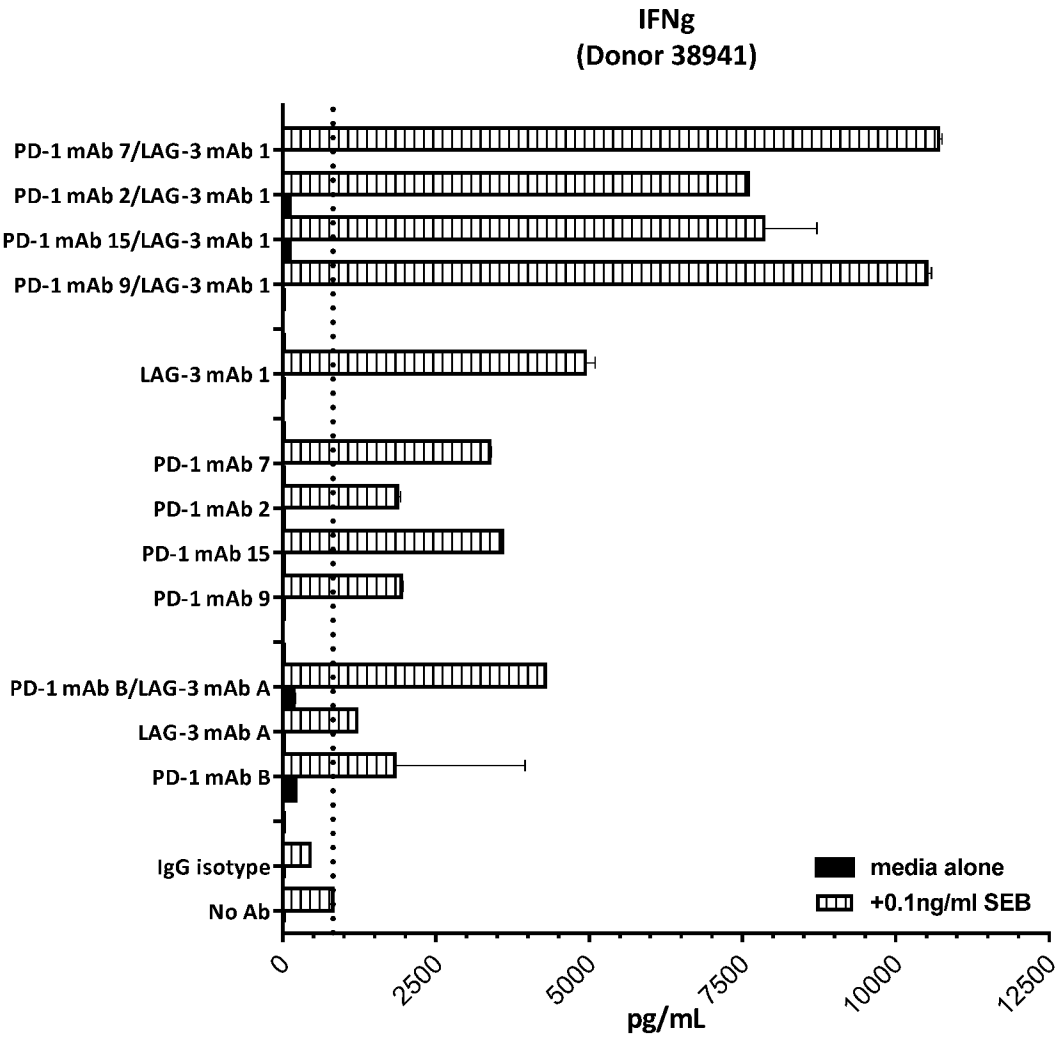
IFNg
(Donor 38941)
PD-1 mAb 7/LAG-3 mAb 1
PD-1 mAb 2/LAG-3 mAb 1
PD-1 mAb 15/LAG-3 mAb 1
PD-1 mAb 9/LAG-3 mAb 1
LAG-3 mAb 1
PD-1 mAb 7
PD-1 mAb 2
PD-1 mAb 15
PD-1 mAb 9
PD-1 mAb B/LAG-3 mAb A
LAG-3 mAb A
PD-1 mAb B
IgG isotype
No Ab
media alone
+0.1ng/ml SEB
0
2500
5000
7500
10000
12500
pg/mL

Figure 14

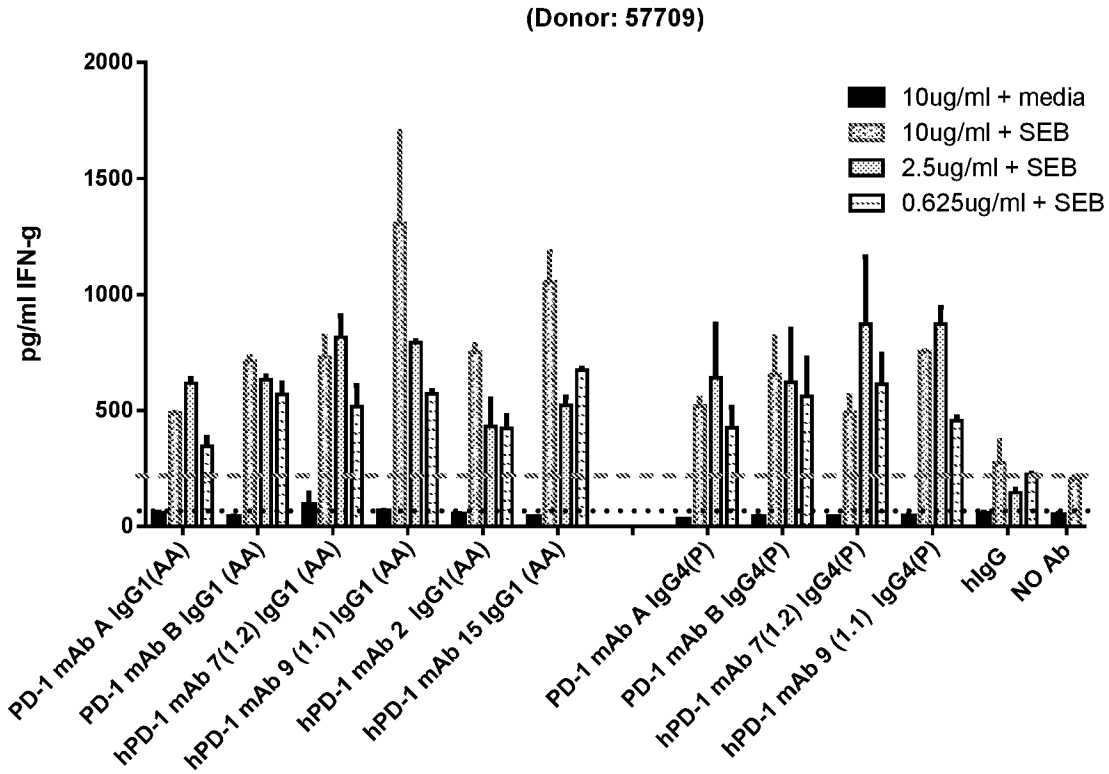
(Donor: 57709)
pg/ml IFN-g
2000
1500
1000
500
0
10ug/ml + media
10ug/ml + SEB
2.5ug/ml + SEB
0.625ug/ml + SEB
PD-1 mAb A IgG1(AA)
PD-1 mAb B IgG1 (AA)
hPD-1 mAb 7(1.2) IgG1 (AA)
hPD-1 mAb 9 (1.1) IgG1 (AA)
hPD-1 mAb 2 IgG1(AA)
hPD-1 mAb 15 IgG1 (AA)
PD-1 mAb A IgG4(P)
PD-1 mAb B IgG4(P)
hPD-1 mAb 7(1.2) IgG4(P)
hPD-1 mAb 9 (1.1) IgG4(P)
hIgG
NO Ab

Figure 15A

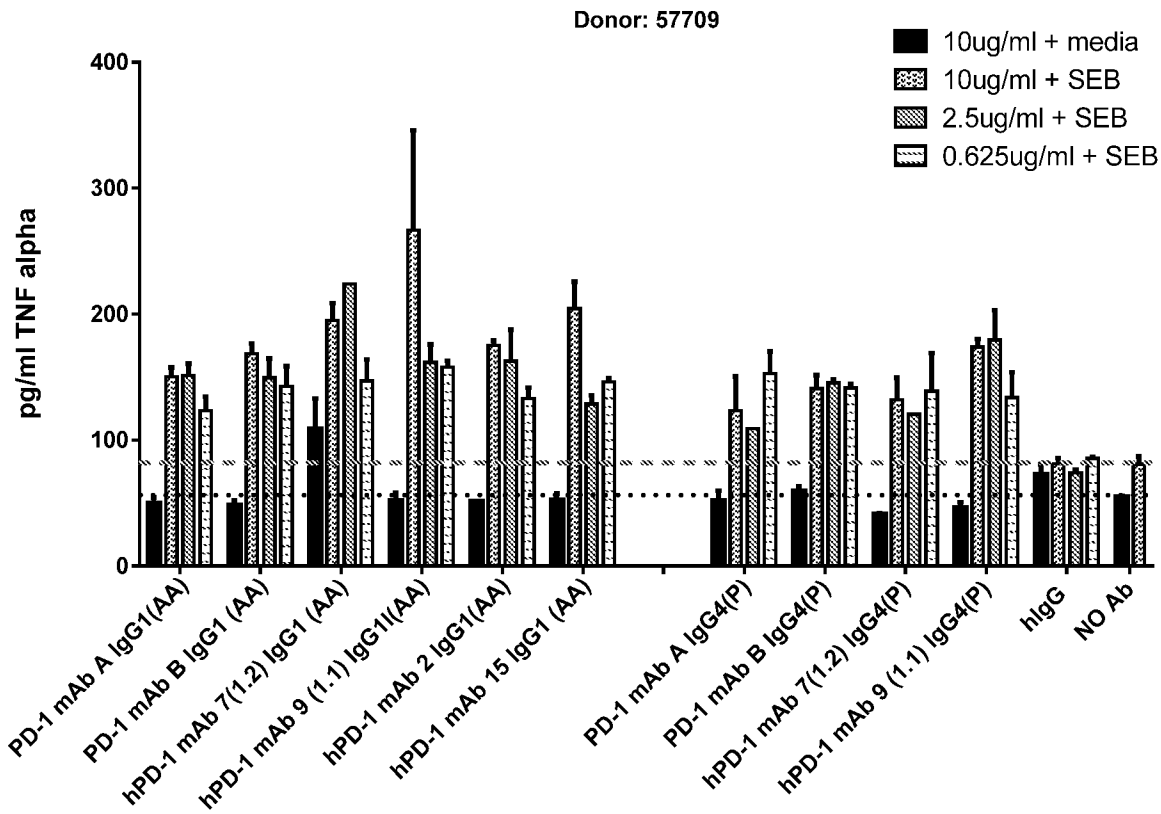
Donor: 57709
10ug/ml + media
10ug/ml + SEB
2.5ug/ml + SEB
0.625ug/ml + SEB
pg/ml TNF alpha
400
300
200
100
0
PD-1 mAb A IgG1(AA)
PD-1 mAb B IgG1 (AA)
hPD-1 mAb 7(1.2) IgG1 (AA)
hPD-1 mAb 9 (1.1) IgG1l(AA)
hPD-1 mAb 2 IgG1(AA)
hPD-1 mAb 15 IgG1 (AA)
PD-1 mAb A IgG4(P)
PD-1 mAb B IgG4(P)
hPD-1 mAb 7(1.2) IgG4(P)
hPD-1 mAb 9 (1.1) IgG4(P)
hIgG
NO Ab

Figure 15B

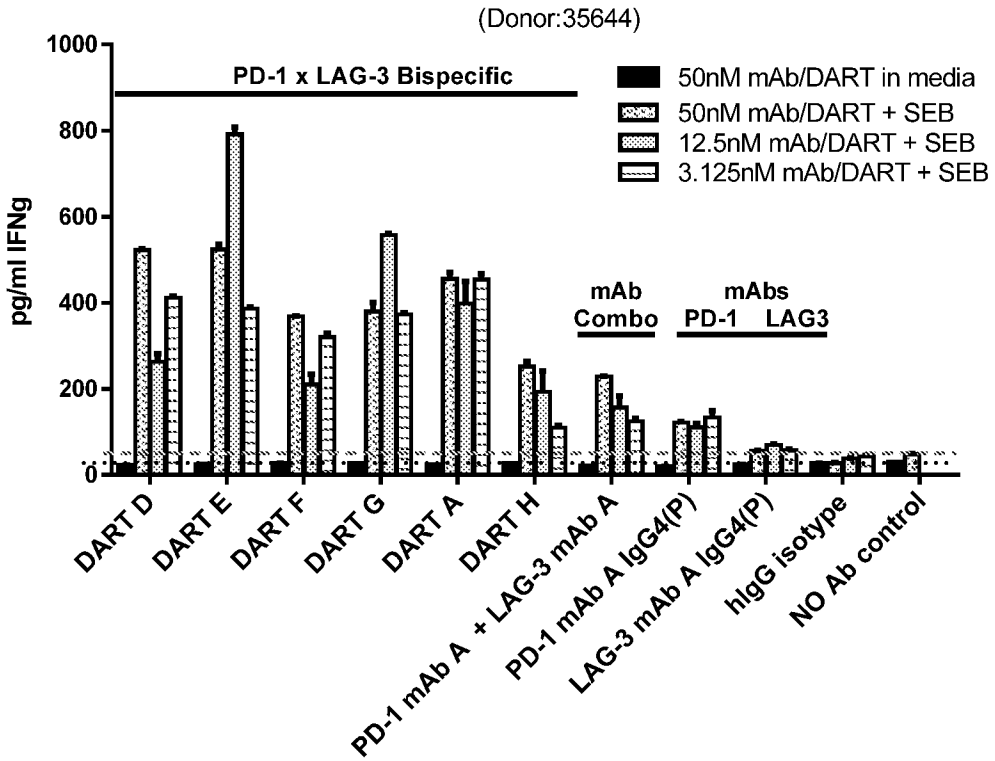

Figure 16A

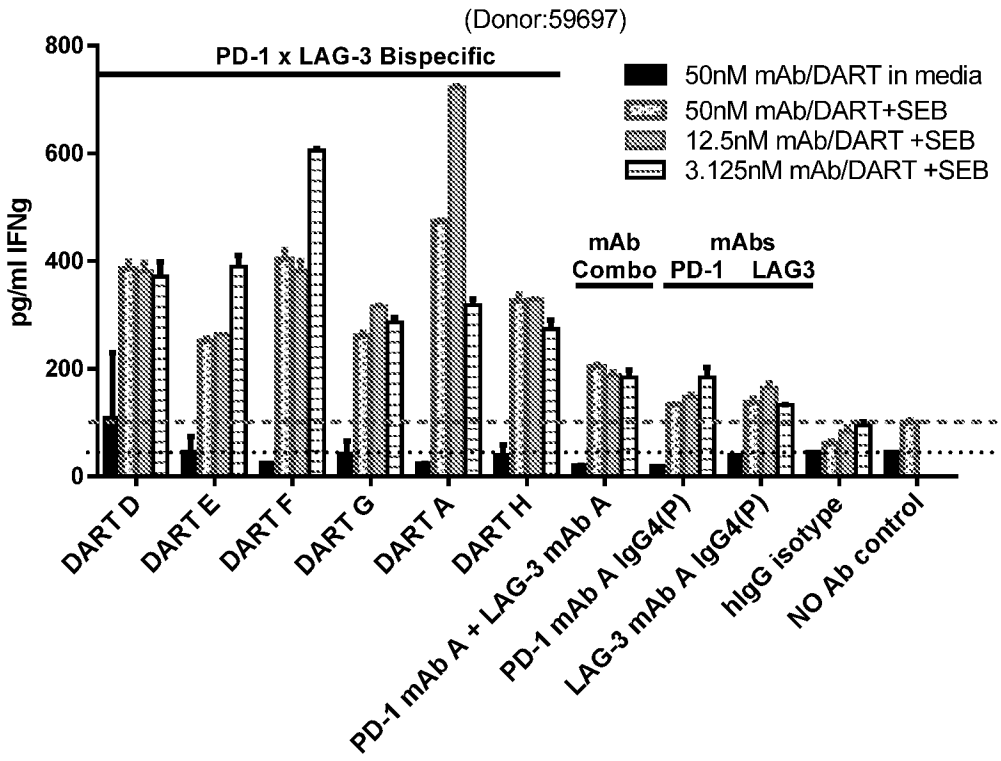

Figure 16B

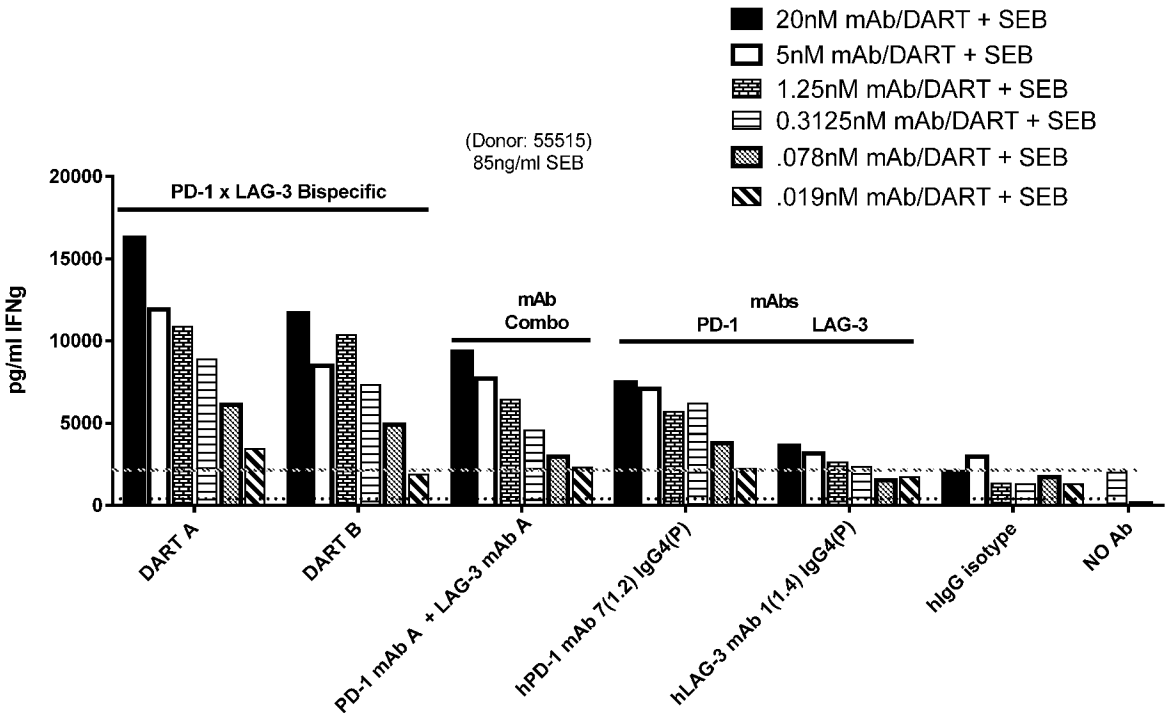

Figure 17A

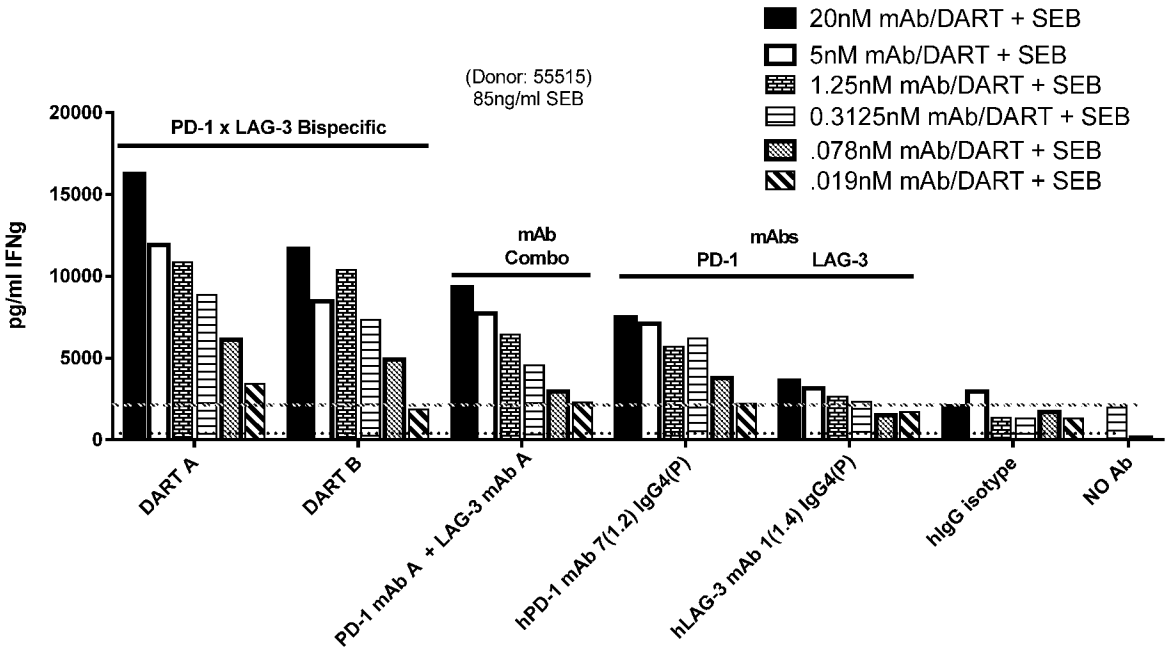

Figure 17B

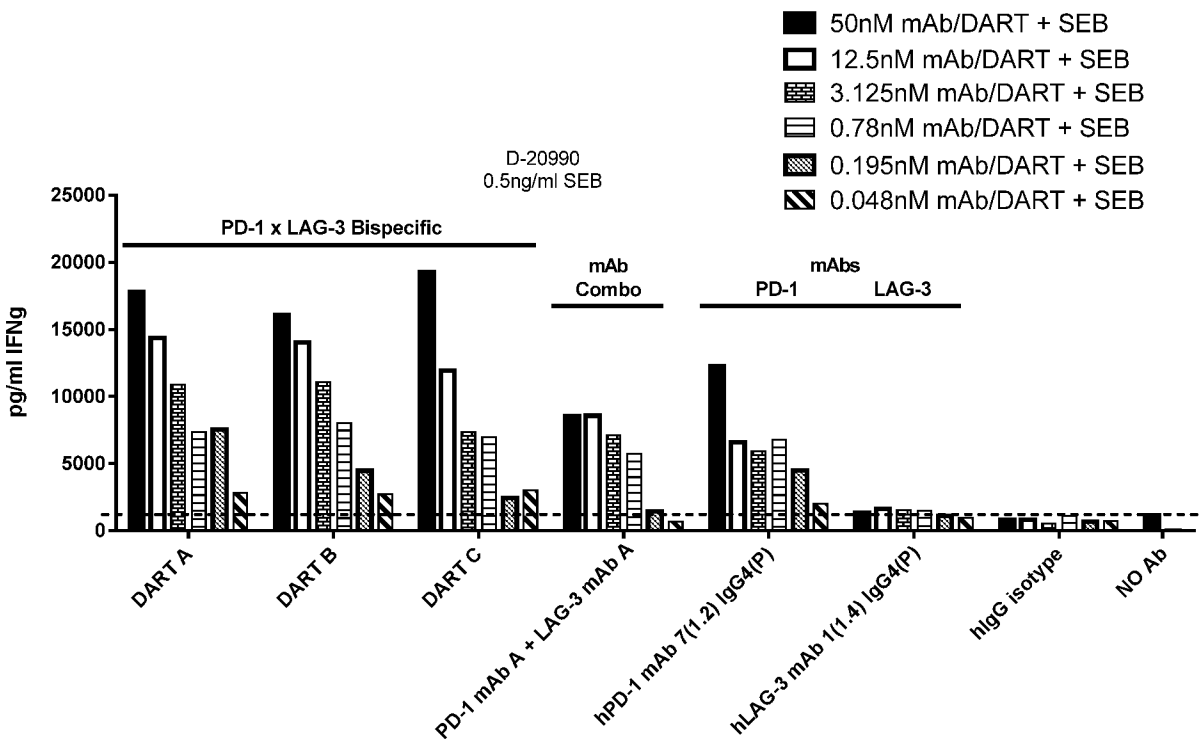
50nM mAb/DART + SEB
12.5nM mAb/DART + SEB
3.125nM mAb/DART + SEB
0.78nM mAb/DART + SEB
0.195nM mAb/DART + SEB
0.048nM mAb/DART + SEB
D-20990
0.5ng/ml SEB
PD-1 x LAG-3 Bispecific
mAb
Combo
mAbs
PD-1
LAG-3
pg/ml IFNg
25000
20000
15000
10000
5000
0
DART A
DART B
DART C
PD-1 mAb A + LAG-3 mAb A
hPD-1 mAb 7(1.2) IgG4(P)
hLAG-3 mAb 1(1.4) IgG4(P)
hIgG isotype
NO Ab

Figure 18A

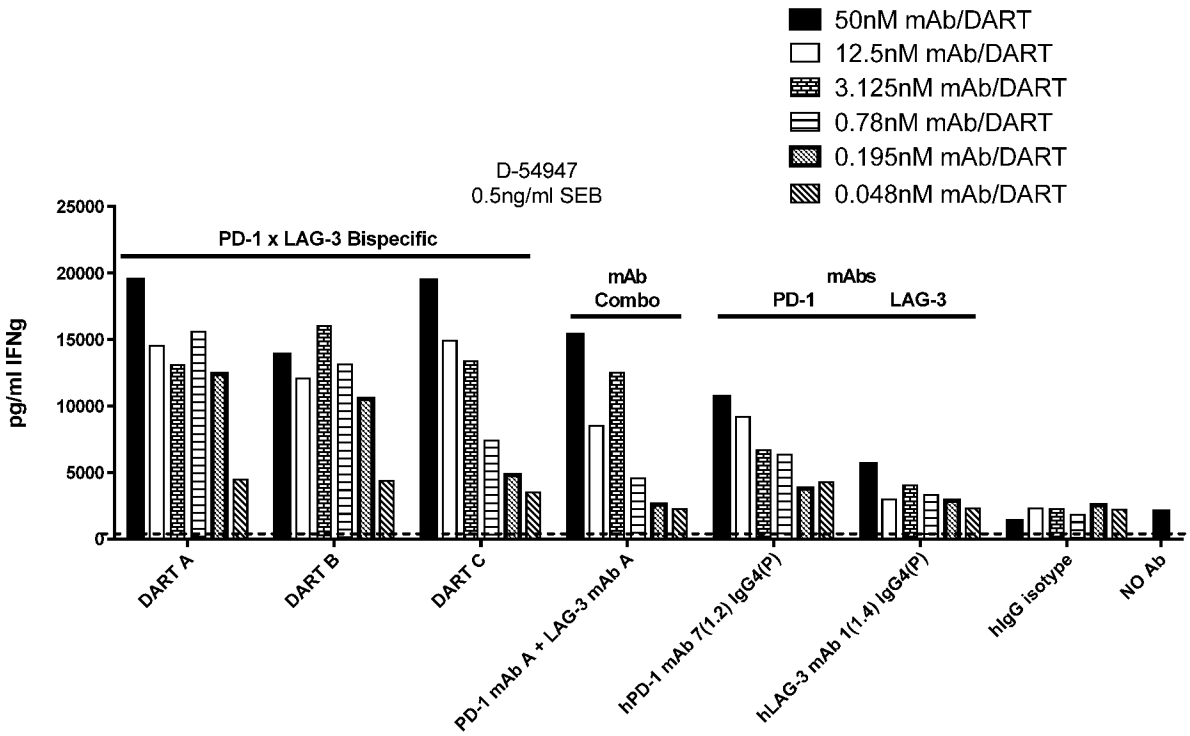
50nM mAb/DART
12.5nM mAb/DART
3.125nM mAb/DART
0.78nM mAb/DART
0.195nM mAb/DART
0.048nM mAb/DART
D-54947
0.5ng/ml SEB
PD-1 x LAG-3 Bispecific
mAb Combo
mAbs
PD-1
LAG-3
pg/ml IFNg
25000
20000
15000
10000
5000
0
DART A
DART B
DART C
PD-1 mAb A + LAG-3 mAb A
hPD-1 mAb 7(1.2) IgG4(P)
hLAG-3 mAb 1(1.4) IgG4(P)
hIgG isotype
NO Ab

Figure 18B

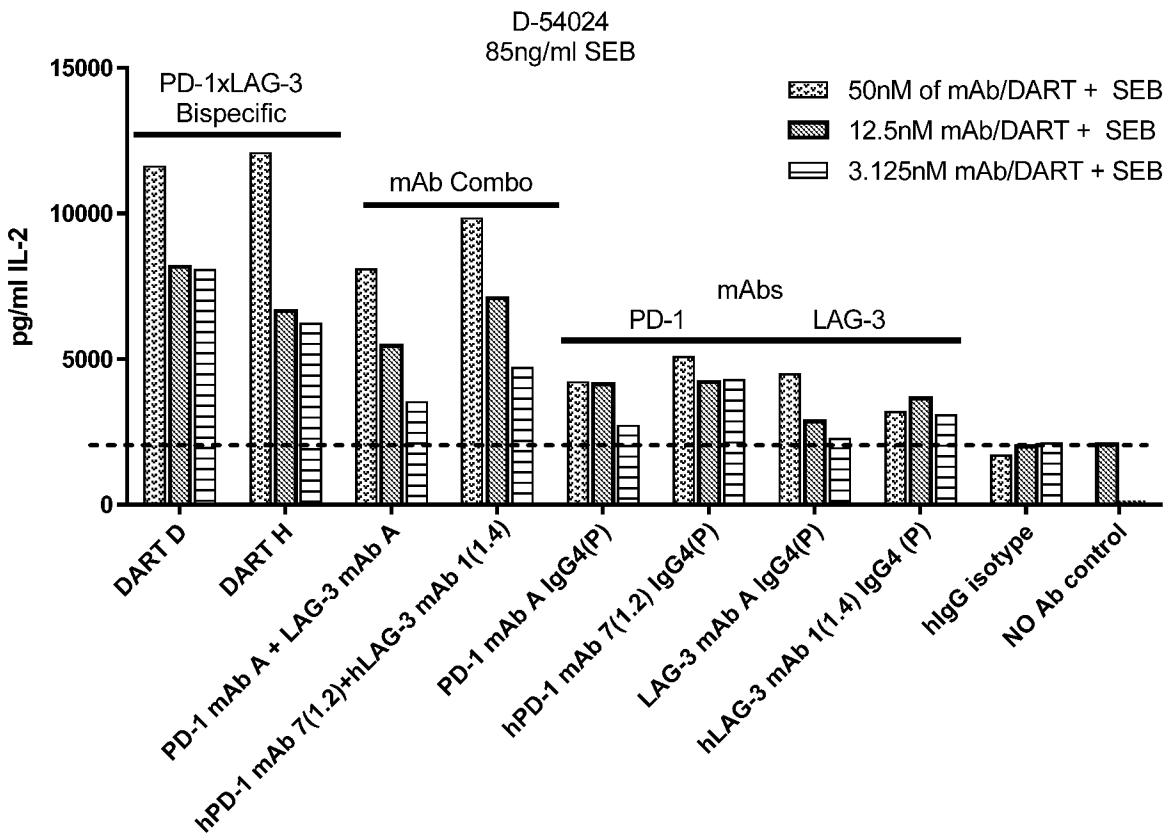
D-54024
85ng/ml SEB
50nM of mAb/DART + SEB
12.5nM mAb/DART + SEB
3.125nM mAb/DART + SEB
pg/ml IL-2
15000
10000
5000
0
PD-1xLAG-3 Bispecific
mAb Combo
mAbs
PD-1
LAG-3
DART D
DART H
PD-1 mAb A + LAG-3 mAb A
hPD-1 mAb 7(1.2)+hLAG-3 mAb 1(1.4)
PD-1 mAb A IgG4(P)
hPD-1 mAb 7(1.2) IgG4(P)
LAG-3 mAb A IgG4(P)
hLAG-3 mAb 1(1.4) IgG4 (P)
hIgG isotype
NO Ab control

Figure 19

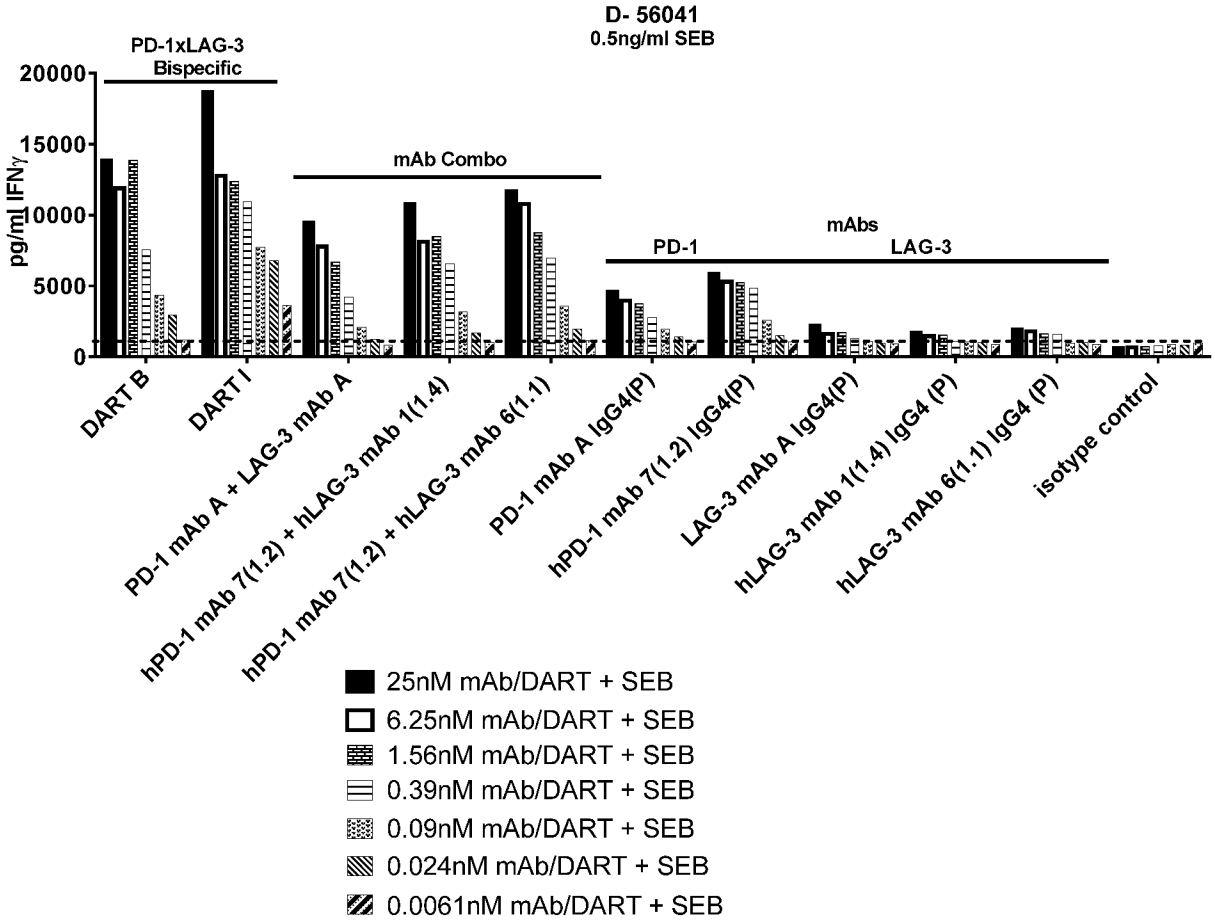
D- 56041
0.5ng/ml SEB
PD-1xLAG-3
Bispecific
mAb Combo
mAbs
PD-1
LAG-3
pg/ml IFNγ
20000
15000
10000
5000
0
DART B
DART I
PD-1 mAb A + LAG-3 mAb A
hPD-1 mAb 7(1.2) + hLAG-3 mAb 1(1.4)
hPD-1 mAb 7(1.2) + hLAG-3 mAb 6(1.1)
PD-1 mAb A IgG4(P)
hPD-1 mAb 7(1.2) IgG4(P)
LAG-3 mAb A IgG4(P)
hLAG-3 mAb 1(1.4) IgG4 (P)
hLAG-3 mAb 6(1.1) IgG4 (P)
isotype control
25nM mAb/DART + SEB
6.25nM mAb/DART + SEB
1.56nM mAb/DART + SEB
0.39nM mAb/DART + SEB
0.09nM mAb/DART + SEB
0.024nM mAb/DART + SEB
0.0061nM mAb/DART + SEB

Figure 20

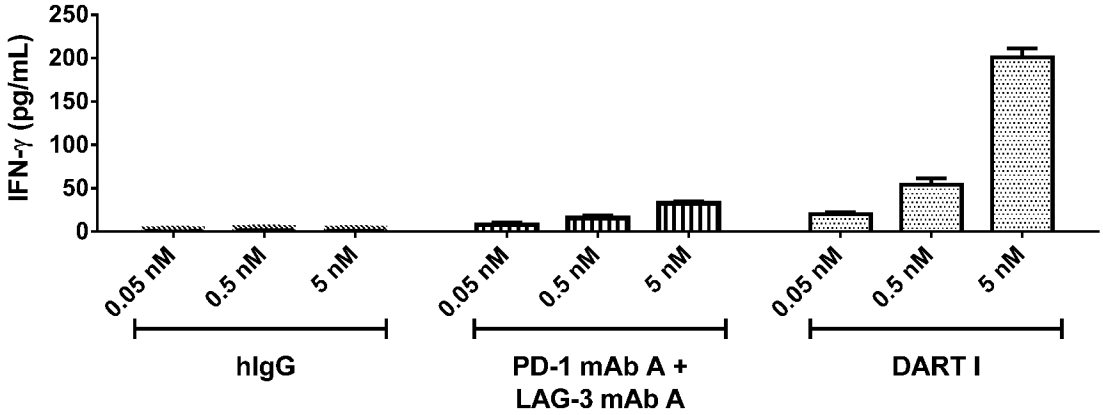

Figure 21A

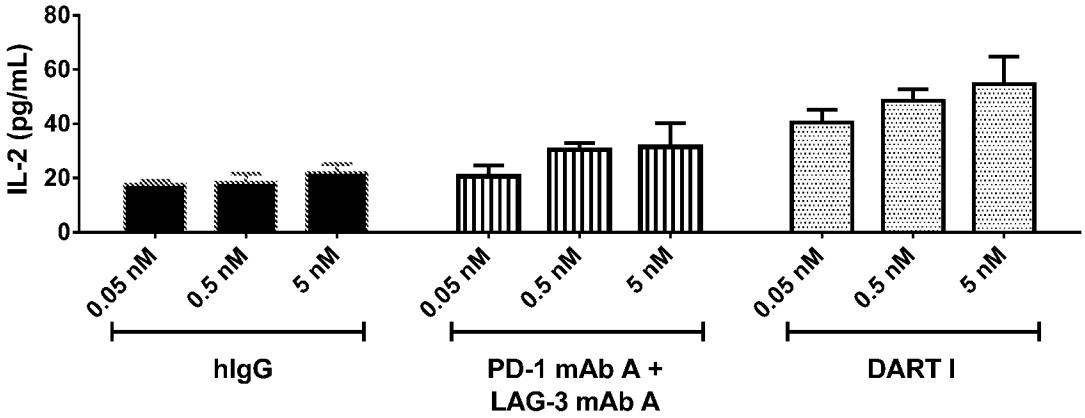

Figure 21B

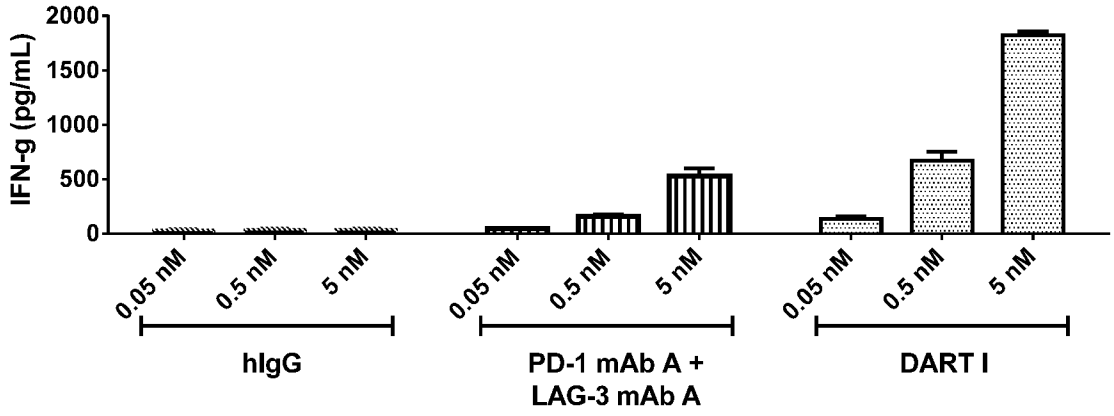

Figure 21C

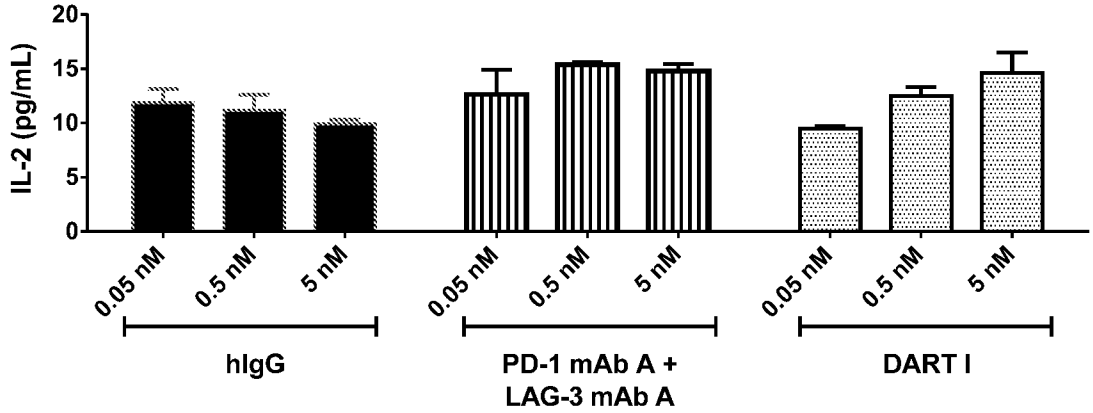

Figure 21D

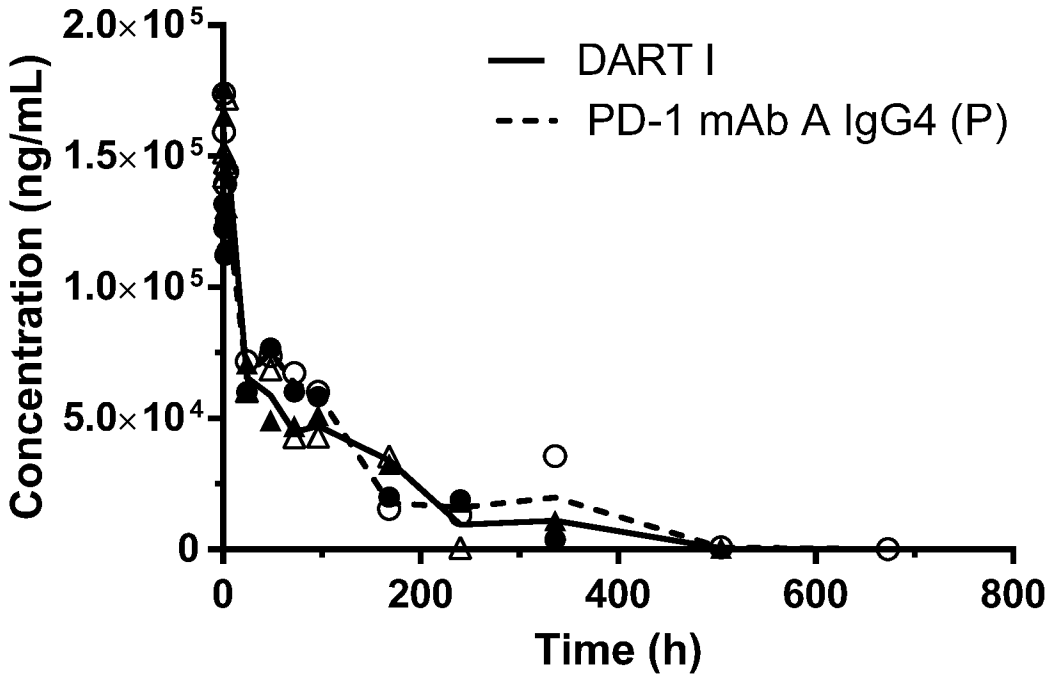
2.0×10^5
1.5×10^5
1.0×10^5
5.0×10^4
0
Concentration (ng/mL)
0
200
400
600
800
Time (h)
DART I
PD-1 mAb A IgG4 (P)

Figure 22

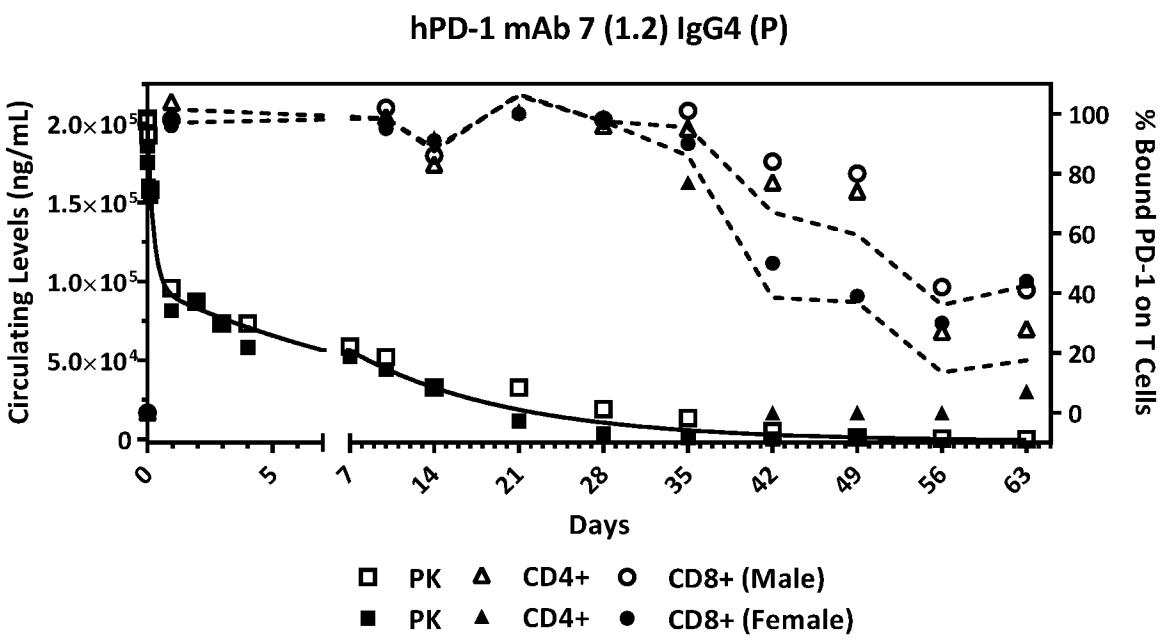

Figure 23A

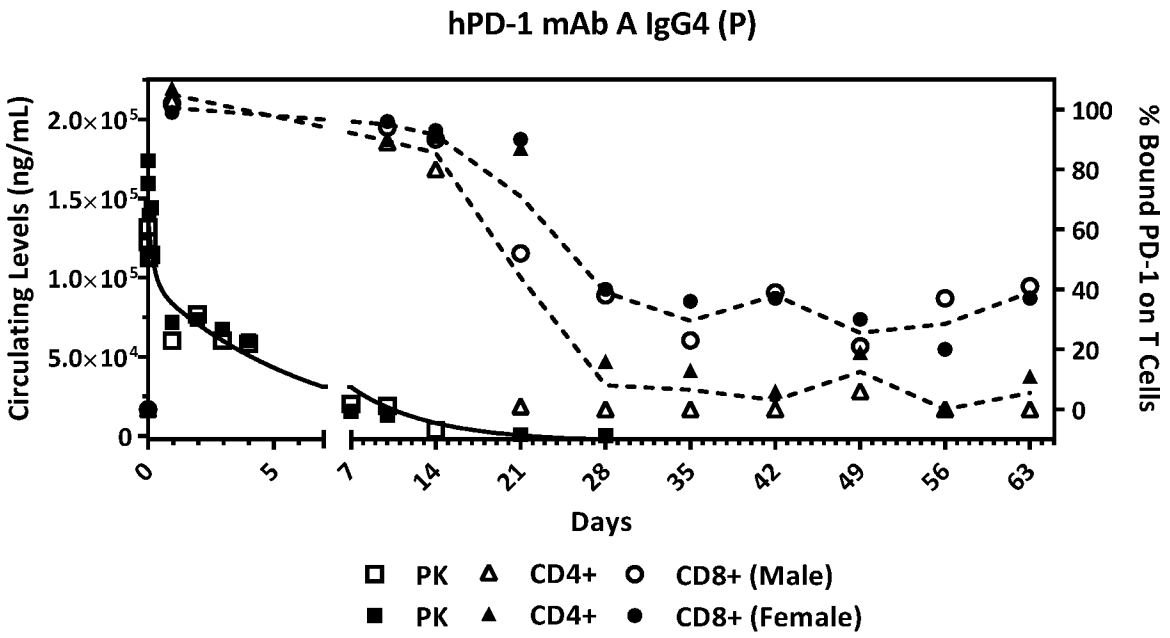

Figure 23B

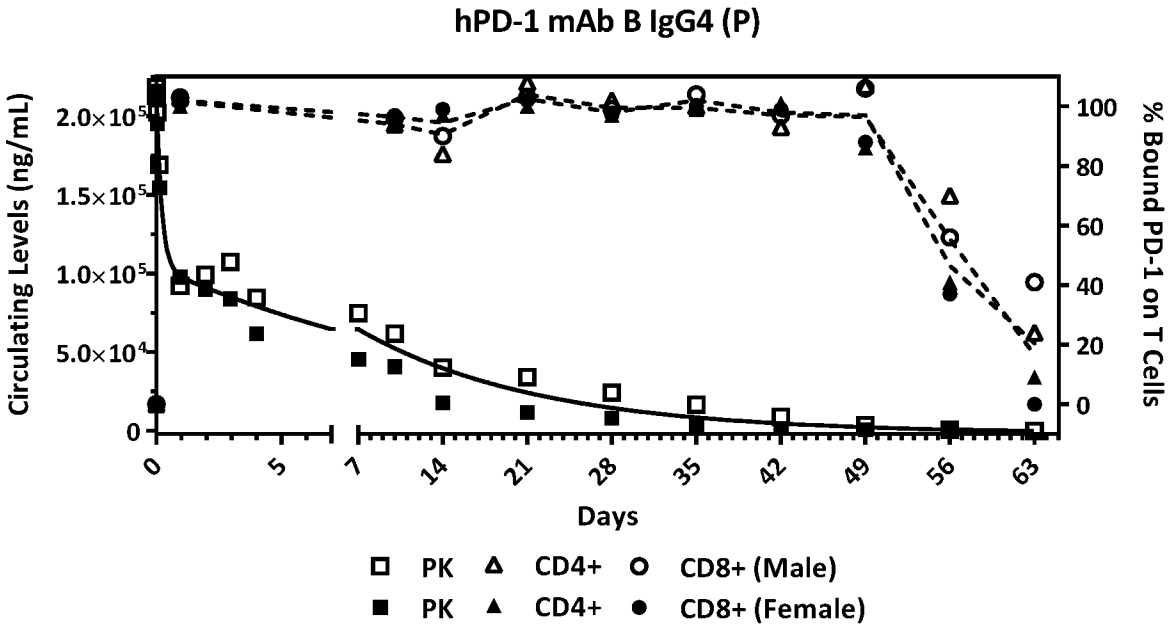
hPD-1 mAb B IgG4 (P)
Circulating Levels (ng/mL)
2.0×10^5
1.5×10^5
1.0×10^5
5.0×10^4
0
% Bound PD-1 on T Cells
100
80
60
40
20
0
0
5
7
14
21
28
35
42
49
56
63
Days
PK
CD4+
CD8+ (Male)
PK
CD4+
CD8+ (Female)

Figure 23C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/044430

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NOs: 79, 81, 93, 95, 147, 149, 151, 153, 155, 179, 181, 184, 186, 250, 251, 264, 265, and 267-269 were searched.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/044430

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 5-27
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/044430

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/00; A61K 39/395; C07K 16/00 (2016.01)
CPC - A61K 39/395; C07K 16/468; C07K 2317/73 (2016.08)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC - A61K 39/00; A61K 39/395; C07K 16/00
CPC - A61K 39/395; C07K 16/468; C07K 2317/73

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 424/13.1; 424/130.1; 530/387.1 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Patbase, Google Patents, PubMed, Google

Search terms used: anti PD-1 human antibody heavy chain light chain variable humanized chimeric

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2015/0079109 A1 (BEIGENE LTD) 19 March 2015 (19.03.2015) entire document	1-4
A	WO 2015/026894 A2 (MACROGENICS, INC.) 26 February 2015 (26.02.2015) entire document	1-4
A	US 2013/0109843 A1 (MSD OSS B.V. et al) 02 May 2013 (02.05.2013) entire document	1-4
A	US 2014/0348743 A1 (ONO PHARMACEUTICAL CO. LTD.) 27 November 2014 (27.11.2014). entire document	1-4
A	US 2014/0234296 A1 (SHARMA et al) 21 August 2014 (21.08.2014) entire document	1-4

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
03 October 2016	24 OCT 2016
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774